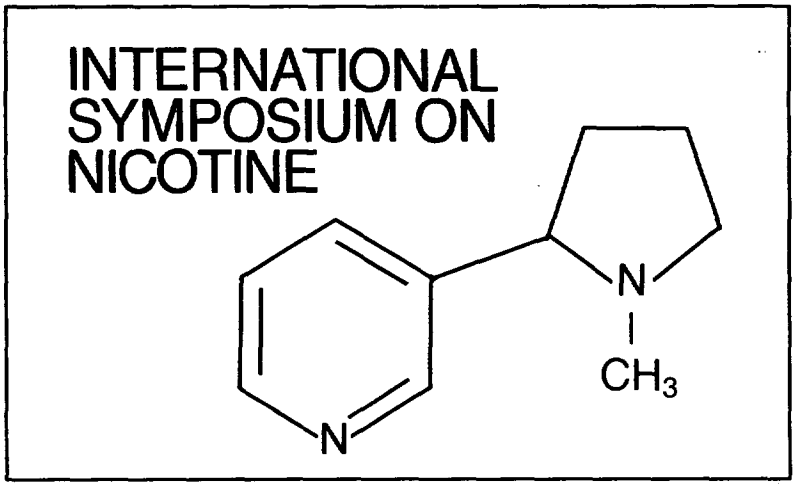
Abstracts

Effects of Nicotine on Biological Systems



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Invited Lectures

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THE ETHNOPHARMACOLOGY OF TOBACCO IN NATIVE SOUTH AMERICA

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The use of tobacco by South American Indians is deeply rooted in their culture and thought. From early pre-Columbian times to the present, tobacco has functioned as an important psychotropic drug for magico-religious, medicinal, and recreational purposes. Native interest in tobacco centers on the nicotine alkaloid it contains. Data culled from about 1,800 sources and pertaining to nearly 300 societies reveal that South American Indians employ six major and several minor means of nicotine application. There exists a close functional relationship between tobacco and shamanism.

The empirical ethnographical data base of nicotine application is compared to the comprehensive literature of experimental clinical studies of tobacco and nicotine. Ritual tobacco use aims to achieve acute nicotine intoxication. The pharmacological effects of the alkaloid on the human body are shown to have informed shamanic therapeutic practices and beliefs.

Closely associated with soil cultivation, tobacco use in the New World is much more recent than shamanism. Thus, it is not the drug that gave origin to shamanic religion but religion than informed the effects of the drug.

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STUDIES ON THE METABOLIC FATE OF THE TOBACCO ALKALOIDS (S)-NICOTINE, (S)-COTININE AND β-NICOTYRINE

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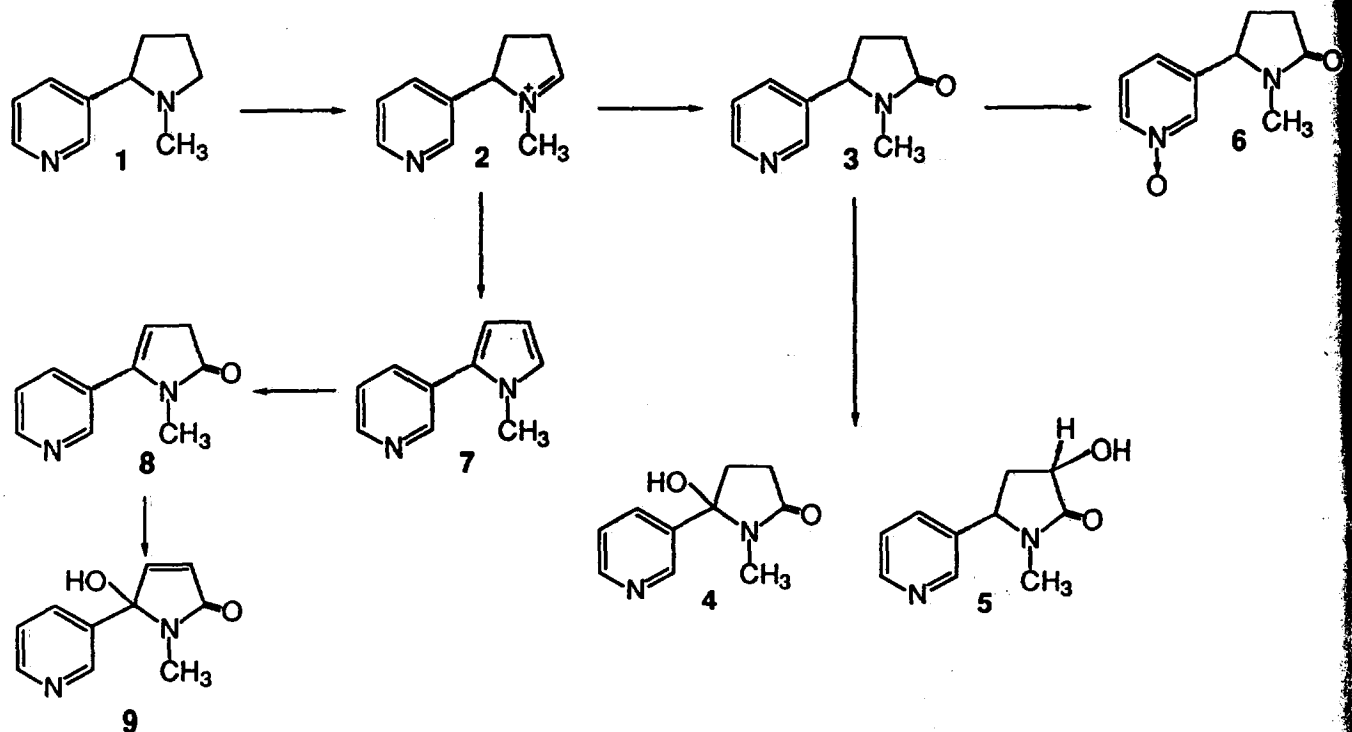
The principal primary *in vitro* metabolic conversion of (S)-nicotine (1) in mammals is catalyzed by cytochrome P-450 and leads to the formation of the corresponding $\Delta^{1',5'}$-iminium species 2, a reactive electrophilic intermediate with bioalkylating potential. Liver aldehyde oxidase catalyzes the conversion of the iminium intermediate 2 to (S)-cotinine (3) which subsequently is oxidized to the isomeric 5'-hydroxycotinine (S)-*trans*-3'-hydroxycotinine and (S)-cotinine-N-oxide (4, 5 and 6, respectively). Alternative primary metabolic routes lead to the formation of (S)-nicotine-N-oxide and nornicotine. Several additional minor metabolites include demethylation and pyrrolidine ring opened products.

The metabolic dependent formation of covalent adducts between radiolabeled (S)-nicotine and microsomal proteins is likely to involve formation of the iminium ion 2. Studies with rodent liver and lung preparations suggest that cytochrome P-450 2 is the primary isozyme responsible for this ring α-carbon oxidation reaction. The reaction pathway preceeds via the regio and stereoselective abstraction of the 5'-*pro-E* proton. Model 2-electron oxidations of the pyrrolidinyl ring of (S)-nicotine failed to provide similar stereochemical selectivity suggesting the formation of a highly ordered complex between this substrate and the active site of the enzyme.

Questions concerning the potential toxicity of the (S)-nicotine iminium species 2 generated in cytochrome P-450 containing tissues which lack aldehyde oxidase prompted more detailed studies on the metabolic fate of this intermediate. Of particular interest was the observation that monoamine oxidase B catalyzes the oxidation of 2 to β-nicotyrine (7). As expected, this electron rich pyrrolic compound proved to be an excellent substrate for liver and lung oxidases. Analysis of β-nicotyrine containing microsomal incubation mixtures by collision induced dissociation HPLC-MS/MS techniques led to the characterization of the unstable aminoenone 8 and one of its autoxidation products, the 5'-hydroxypyrrolinone 9. The mechanistic features and potential toxicological significance of these transformations will be considered.



Metabolic transformation of (S)-nicotine initiated by the cytochrome P-450 catalyzed oxidation at C-5'.

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OXIDATIVE METABOLISM OF NICOTINE *IN VIVO*

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Nicotine undergoes a variety of metabolic reactions involving oxidation, most of which result in transformations of the pyrrolidine ring. The major route of metabolism in most mammalian species involves oxidation of the 5'-carbon atom to give the γ-lactam derivative cotinine. Oxidation of the N-methyl group results in demethylation to nornicotine, which is a minor metabolite in humans. Nicotine also undergoes oxidation of the pyrrolidine nitrogen atom to give a diasteriomeric mixture of N-oxides. Cotinine is extensively metabolized in humans, with only 10-20% being excreted unchanged in urine. It appears that the major urinary metabolite of cotinine is trans-3'-hydroxycotinine which results from oxidation adjacent to the carbonyl. Formation of this metabolite in humans has been shown to be highly stereoselective. Cotinine is also metabolized by N-oxidation of the pyridine nitrogen atom, but cotinine N-oxide is a minor metabolite in humans, accounting for only a few percent of the nicotine absorbed by smokers. Other metabolites resulting from oxidative degradation of the pyrrolidine ring have been reported, but the pathways by which they are formed are not well understood, and their quantitative importance in humans is unknown.

In this paper the various oxidative pathways will be discussed, with emphasis on quantitative and stereochemical aspects of nicotine metabolism in humans. Applications of metabolic data in clinical pharmacologic studies will also be discussed.

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CONJUGATION PATHWAYS IN NICOTINE METABOLISM

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The major metabolic pathways in the phase 1 metabolism of nicotine are C-oxidation to cotinine, which in turn is extensively metabolized to *trans*-3´-hydroxycotinine, and the N-oxidation to the two diastereomers of nicotine-N´-oxide. Together with nicotine these major phase 1 metabolites account for about 65 % of the ingested dose.

Glucuronidation is among the most frequently used means by which humans produce polar metabolites of xenobiotics for excretion. Our recent studies have revealed that nicotine, cotinine and *trans*-3´-hydroxycotinine form glucuronic acid conjugates (phase 2 metabolism), which are excreted in the urine.

Characterization of the conjugates, investigation of the conditions for the enzymatic hydrolysis of the glucuronides and an indirect analytical procedure will be presented.

The major phase 1 and phase 2 metabolites of nicotine have been quantified in two studies. In one study, the 24-hours excretion of these metabolites were determined in habitual users of moist snuff, and in the other study the total amount of these metabolites excreted over 94 hours after intravenous infusion of nicotine to a group of abstinent tobacco users were determined.

Our results show that by measuring excretion of nicotine and its major metabolites, 80-90 % of the dose of nicotine could be quantified in human urine.

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RELEVANCE OF NICOTINE-DERIVED N-NITROSAMINES IN TOBACCO CARCINOGENESIS

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Human exposure to N-nitroso compounds is widespread. Chewing tobacco, snuff, and tobacco smoke contain tobacco-specific N-nitrosamines (TSNA) far in excess of concentrations of nitrosamines reported in other consumer products. TSNA are formed from nicotine and the minor Nicotiana alkaloids during tobacco processing and smoking. They induce benign and malignant tumors of the upper aerodigestive tract, lung, liver and/or exocrine pancreas in mice, rats and hamsters. The epidemiological studies on snuff dippers strongly support the contention that TSNA contribute significantly to the increased cancer risk of tobacco consumers. The known carcinogens in snuff are certain aldehydes, polonium-210, polynuclear aromatic hydrocarbons, and TSNA which are the major carcinogens in snuff. A mixture of N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), swabbed daily on the oral tissue of rats (total doses: 1.6 mM/kg)induced a significant incidence of oral tumors. The lifetime exposure of a snuff dipper to NNN and NNK amounts to approximately 0.4 mmol/kg. In addition, endogenous formation of TSNA during tobacco usage is likely.

Biochemical studies strongly support the contention that TSNA make a significant contribution to the carcinogenic risk of tobacco chewers and smokers. NNN and NNK require metabolic conversion to active species which react with cellular components including DNA. In human tissue explants of oral cavity, lung and liver, NNN and NNK are metabolically activated in the same way as in mice, rats and hamsters. In snuff dippers and smokers, TSNA form also globin adducts, which are studied as markers of exposure to NNN and NNK. The concentration of the TSNA adducts in the blood of snuff dippers exceeds that expected from the TSNA content of the consumed tobacco. This finding supports the concept that TSNA are also formed endogenously in tobacco consumers. Compared to nonsmokers, cigarette smokers have a higher potential for endogenous N-nitrosamine formation as is reflected in their higher urinary excretion of N-nitrosoproline and N-nitrosothioproline.

These studies are supported by Grants No. 29580 and 44377 from the United States National Cancer Institute.

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Effects of Nicotine on Insulin: Actions and Implications

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In 1988, we reported that nicotine decreases plasma levels of insulin in a dose-response fashion. More specifically, two weeks of continuous administration by osmotic minipump of 6 mg and 12 mg nicotine/kg body weight/day to rats results in significant decreases in plasma insulin. Recently, we found that plasma insulin increases after cessation of this type of nicotine administration, slightly overshooting control values for a few days. We have continued to explore the effects of nicotine on insulin. The presentation will review our findings regarding nicotine's effects on insulin and will present new data indicating that nicotine administration somewhat decreases pancreatic levels of insulin and slightly increases levels of insulin in the hypothalamus. The implications of these findings for nicotine's effects on body weight, specific food preferences, energy expenditure, and energy efficiency will be discussed. Moreover, the possible role of insulin as a modulator of nicotine reinforcement will be discussed.

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EFFECT OF NICOTINE ON PULMONARY DEFENCE SYSTEMS.

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To protect itself against inhaled noxious agents the respiratory tract employs a vital defence system containing several organelles: the mucociliary system adsorbs and removes inhaled materials; sensory neuroreceptors control respiration: shallow breathing impedes penetration of agents into deeper lung zones, coughing removes particle-laden mucus; bronchial smooth muscle, by causing bronchoconstriction, may limit the depth of particle penetration and will increase the linear velocity of air, thereby improving cough efficiency. Mucosal blood vessels with their dense innervation determine mucosal thickness and mucosal liquid flow, they also import inflammatory cells and mediators into mucosal tissues, initiating restitutive processes following mucosal damage.

Nicotine given acutely affects a number of these systems. Nicotine increases mucus secretion both after local application and after systemic uptake. Local effects are observed at high concentrations of nicotine reached during the smoking process. Local effects appear to be mediated by nicotinic receptors in the end organ or by neural pathways not involving sodium channels nor release of endogenous neuropeptides. There are added secretory effects of systemic nicotine which are mediated through muscarinic receptors. These secretory effects of nicotine are complemented by irritant effects on sensory receptors causing cough. Nicotine further promotes particle clearance by increasing the beat frequency of respiratory tract cilia, an effect which is independent of the increase in mucus secretion. Nicotine increases airway smooth muscle tension through cholinergic neural pathways and through muscarinic receptors in the end organ. There is evidence that part of the effect is caused by axon reflexes following irritation of sensory C-fibres with release of neuropeptides which is in contrast to effects of nicotine on airway secretion where endogenous neuropeptides play a minor role if any. Nicotine by itself (given locally) can increase blood vessel diameter and permeability by its effect on peptide-containing nerves but these effects are opposed by systemic nicotine causing powerful vasoconstriction through the release of catecholamines from sympathetic nerve endings and from the adrenal medulla. Summarizing, mucus secretion, cough, bronchoconstriction and increased ciliary beating are likely to improve mucociliary clearance whereas the balance of vascular effects is difficult to predict. Vasodilation would augment, vasoconstriction inhibit mucosal oedema and inflammation.

Chronic effects of nicotine on submucosal glands include a significant shift from serous to mucous acini, in some cases outright gland hypertrophy. This shift in gland structure is analogous to the shift seen in chronic bronchitis or after chronic β-adrenergic stimulation and is undesirable because it may contribute to the chronic hypersecretory state seen in (and adding to) obstructive disease. Thus, although many short term effects of nicotine aid mucociliary clearance long term application is likely to impede clearance mechanisms by causing obstruction and by adding to the mucus load the system has to cope with.

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A NEW ROLE FOR NICOTINE: SELECTIVE INHIBITION OF THROMBOXANE FORMATION BY DIRECT INTERACTION WITH THROMBOXANE SYNTHASE

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Animal cells can convert arachidonic acid into prostaglandins, thromboxane (TX), and leukotrienes (1-6). These locally produced mediators of inflammatory and hypersensitivity reactions have been implicated in several clinically important disease processes. TX, the major oxygenated arachidonic acid metabolite of macrophages, is the most potent vasoconstricting and proaggregatory molecule known and has also been related to host defense mechanisms. We have studied effects of nicotine on TX formation using cultured macrophage-like cells, microsomal assays, and purified TX synthase. In intact macrophage-like cells nicotine, cotinine, and methylnicotine, at submicromolar concentrations, inhibited the rate of conversion of endoperoxide PGH_2 into TX but not into prostacyclin. This indicated that nicotine selectively inhibited TX synthase at concentrations that are readily observed in the circulation of smokers. Microsomal assays revealed that nicotine decreased the maximal velocity (V_{max}) of TX synthase without affecting the apparent affinity of the enzyme for its substrate (K_M). No effect of nicotine on kinetic parameters of prostacyclin synthase could be observed. To elucidate the molecular mechanism of nicotine's effect we obtained difference spectra using purified TX synthase. The difference spectra revealed that nicotine directly interacted with the enzyme presumably by binding of the nitrogen of the nicotine ring structure to the iron of the cytochrome p-450 component of TX synthase.

Conclusions:
When considered together we have obtained evidence for a new mechanism of action of nicotine. Our results raise the possibility that the effects of nicotine and cotinine on TX synthase are related to smoking-induced changes in blood coagulation, chronic inflammatory lung disease, and impaired host defense all of which have been demonstrated in smokers.

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NEW NICOTINIC AGONISTS, ANTAGONISTS, AND ION CHANNEL BLOCKERS TO PROBE MULTIPLE SITES OF NICOTINE'S ACTION IN BRAIN.

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With the use of (-)-^{3}H-nicotine and, particularly, ^{3}H-1-methyl-2-(3-pyridyl)-azetidine, a more potent nicotine analogue, Scatchard analyses have revealed the existence of two distinct receptor binding sites with K_d values of 0.7 pM and 2 nM and B_{max} values of 3 and 25 fmoles mg protein; wheras, with either ^{3}H-acetylcholine or ^{3}H-methylcarbamylcholine, a pure nicotinic agonist, only the lower affinity binding site is observed. In an effort to examine the mechanism whereby mecamylamine and other ion channel blockers antagonize the central actions of nicotine, binding studies were performed on rat brain membranes with ^{3}H-mecamylamine. Scatchard analysis revealed the presence of two sites with K_d values of 96nM and I um and B_{max} values of 7 and 30 pmoles/ mg respectively. With a series of mecamylamine and pempidine analogues a good correlation was observed between their affinity for the ^{3}H-mecamylamine binding site and their ability to prevent nicotine-induced seizures in mice and prostration produced by intraventricular administration of nicotine to rats. The ^{3}H-mecamylamine site was inhibited by mM concentrations of monovalent cations and sub- mM concentraions of Ca and other cations; and it was completely inhibited after exposure to high T, trypsin, and detergents. Although mecamylamine does not compete for ^{3}H-nicotine binding, nicotine and its analogues exhibit a high affinity for the ^{3}H-mecamylamine binding site The findings suggest that nicotine may be acting both at the nicotinic recognition site and the associated ionic channel. Structure-activity studies with various carbamate esters of choline and other alkylamininoalkyl and heterocyclic amino alcohols reveal that the addition of alkyl substituents on the carbamyl N of choline and other amino alcohols abolishes muscarinic cholinergic properties while enhacing nicotinic properties. Replacement of the methyl group of methylcarbamylcholine by aromatic, heterocyclic, and aliphatic substiutents converts the compounds into pure nicotinic antagonists. Examples of effective nicotinic antagonists are phenylcarbamylcholine, quinuclidinylmethyl carbamate, and benzoylcholine. The specificity of methylcarbamylcholine and the substituted carbamte esters of choline for the nicotinic recognition site was established by demonstrating their very low affinity for the muscarinic cholingergic site (as measured by ^{3}H-quinuclidinylbenzilate binding) and the inability of the agonists to stimulate phosphoinositide (PI) turnover or the antagonists to inhibit carbamylcholine-stimulated PI turnover.

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MECHANISMS UNDERLYING THE PROTECTIVE EFFECTS OF CHRONIC NICOTINE TREATMENT AGAINST DEGENERATION OF CENTRAL DOPAMINE NEURONS BY MECHANICAL LESIONS

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Based on the negative association between smoking and Parkinson's disease independent of other associated factors the possible antiparkinsonian activity with nicotine has been analyzed by studing if chronic nicotine treatment via minipumps protects nigrostriatal neurons against retrograde and antrograde degeneration following a partial hemitransection 2 weeks earlier at the mes-diencephalic level. Catecholamine (CA) fluorescence histochemistry, tyrosine hydroxylase (TH) immunocytochemistry and biochemical analysis of CA have been performed involving also image analysis and tests on striatal dopamine (DA) function including an analysis of striatal blood flow and metabolism.

The lesion-induced disappearance of TH immunoreactive (IR) cell body and dendritic profiles in the substantia nigra was significantly counteracted in the nicotine treated animals especially with regard to dendritic profiles. Serum nicotine levels were in the order of 50 ng/ml. Protective effects were also observed on DA nerve terminals within the neostriatum. The morphometrical analysis is compatible with an ability of chronic nicotine treatment to partly protect nigrostriatal DA neurons against degenerative changes induced by the partial hemitransection. Functional experiments also demonstrated that the chronic nicotine treatment could eliminate the asymmetry in striatal glucose utilization and blood flow seen following the partial hemitransection. The protective effects of nicotine on the lesioned nigrostriatal DA neurons and possibly on the striatal nerve cells may be related to a desensitization of excitatory nicotinic cholinoceptors located on nigral DA nerve cells and on striatal nerve cells. According to this hypothesis chronic nicotine treatment should lead to a marked and preferential reduction of DA utilization in surviving forebrain DA nerve terminal systems after a di-mesencephalic hemitransection. In agreement it was found that this chronic nicotine treatment markedly and differentially reduces the DA utilization in surviving DA nerve terminal systems of the nucleus caudatus putamen, nucleus accumbens and tuberculum olfactorium. The desensitization of excitatory nicotinic cholinoceptors located on nigral and ventromedial tegmental DA nerve cells and/or on DA forebrain terminals probably leads to a reduced firing rate in the nigral cells and a reduced local release of DA in the forebrain and thus to reduced energy demands. Thus, chronic nicotine treatment may have a role in the treatment of degenerative disorders such as Parkinson's disease and Alzheimer's disease, since desensitization may take place also in nicotinic receptors located on cholinergic neurons, severely affected in Alzheimer's disease.

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NICOTINE: A UNIQUE PSYCHOACTIVE DRUG

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Nicotine in tobacco smoking concentrations is a remarkable psychoactive agent. A wide variety of complex stimulant and depressant effects are observed in animals and humans that involve the central and peripheral nervous, cardiovascular, endocrine, gastrointestinal, and skeletal motor systems. The EEG activating effects of small doses of nicotine occur in intact as well as brainstem transected animals. These effects are directly on brainstem neuronal circuits. However, stimulation of peripheral afferents and release of catecholamines and possibly other neurotransmitters and modulators (serotonin, histamine) enhances the direct central effects of nicotine. The EEG activating effects of nicotine result in behavioral arousal. Nicotine increases the amount of REM sleep, an effect also seen with arginine vasopressin. Nicotine and tobacco smoking dramatically reduce phasic stretch reflexes such as the patellar reflex. Both central and peripheral mechanisms are involved. Conditioned avoidance behavior is selectively depressed by nicotine. Nicotine produces profound neuroendocrine changes, including elevated plasma catecholamines, glucose, glucacon, cortisol, insulin, and vasopressin. Considerable individual variability occurs in response to nicotine, suggesting important pharmacogenetic factors.

In the past two years we have been concentrating on the effects of smoking tobacco with different nicotine content on the topographic EEG and Hoffmann (H) reflex. Four groups of normal tobacco smoking adults who had been deprived of tobacco for 12 hours were studied before and after smoking. Each of the subjects smoked a zero, low, high nicotine research or commercial tobacco cigarette. The subjects were not told precisely what they were smoking. Standard 16 channel quantitative topographic EEG recordings were made. Heart rate was recorded on the 17th channel of the polygraph. Arterial blood pressure was measured by auscultation. Smoking the nicotine free cigarette produced no change in mean blood pressure or heart rate. In contrast, smoking any of the nicotine containing cigarettes increased mean blood pressure and heart rate. Smoking any of the four different types of cigarettes increased total alpha EEG activity, although there were marked individual differences. Only after smoking nicotine containing cigarettes was there a mean decrease in $alpha_1$ (7.8-10 Hz) power and an increase in $alpha_2$ (10.2-12.4 Hz) power in the EEG, not only in the occipital areas but also more diffusely throughout the cerebral cortex. There were no consistent mean changes in EEG delta, theta, or beta activity. Individual differences were marked, irrespective of the nicotine content of the cigarette smoked. Two major trends were noted after tobacco smoking: 1) a more diffuse cortical distribution of $alpha_2$ activity and 2) an increase in $alpha_2$ activity related to the nicotine content of the cigarette smoked. Whether similar effects are observed in nondeprived tobacco smokers remains to be determined.

The H reflex is the electrophysiological equivalent of a stretch reflex that allows one to easily measure its recovery cycle in man. It was postulated that the H reflex recovery cycle would be prolonged by tobacco smoking. Normal adult volunteers were the subjects of this study. All were tobacco smokers. They were asked not to smoke for 12 hr prior to testing. The H reflex and its recovery cycle were obtained before and just after smoking on different days one nonfiltered, nicotine free, low, or high nicotine containing tobacco cigarette. After smoking, the high nicotine cigarettes, the subjects showed a clear cut reduction of the H reflex, especially phase III-early inhibition. Individual differences were marked. Smoking low nicotine containing tobacco had only a small effect on the H reflex and smoking nicotine free cigarettes had no effect at all. The data obtained are consistent with evidence in animals that nicotine and tobacco smoke stimulate Renshaw inhibitory neurons in the spinal cord, either directly or indirectly.

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Effects of Nicotine on Brain Metabolism

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Interest in the biochemical and anatomical mechanisms that are involved in the actions of psychoactive drugs, including nicotine, has led to studies on distributions of the functional responses to these drugs. Autoradiographic approaches that have been used in these studies include measurement of rates of cerebral blood flow and glucose utilization. The 2-deoxy-D-[1-^{14}C]glucose method of Sokoloff has been used to map and quantitate effects of various psychoactive drugs in the rat brain (1). The method allows simultaneous measurement of the regional cerebral metabolic rate for glucose (rCMRglc), an index of functional activity, throughout the central nervous system. It can provide information about the initial sites of drug interactions, and about the sites of secondary effects propagated via afferents to brain areas remote from the initial interactions.

The deoxyglucose method has been used to delineate the effects of nicotine treatments in the brain of the Fischer-344 rat (2,3). In rats subjected to acute systemic treatments with l-nicotine, the drug produces stimulation primarily in brain areas reported to contain specific binding sites for [^{3}H]nicotine, indicating that the sites are true receptors, which are linked to functional activity. Nicotine effects on rCMRglc are observed at doses of nicotine which are discriminated by the rat and which produce behavioral and physiological effects. The stimulation is transient, and is antagonized by mecamylamine. The brain areas affected include limbic structures, components of the visual system, brain stem nuclei which are important in cardiovascular reflexes, and several areas involved in motor function. The distribution of the *in vivo* effects of nicotine on rCMRglc implicates various brain regions in the behavioral and physiological effects of nicotine.

Chronic treatment of rats with nicotine produced rCMRglc effects which vary among brain regions (4). The ventral tegmental area, some components of visual pathways, the cerebellum, and vestibular nuclei show tolerance to nicotine challenge in rats given chronic nicotine. However, no regions shows sensitization to nicotine challenge.

The deoxyglucose method has been adapted for human studies with the use of 2-deoxy-2-[^{18}F]fluoro-D-glucose and positron emission tomography (PET) scanning. Using these procedures, the acute effects of euphorigenic treatments with morphine and cocaine have been studied in human volunteers (5,6). Ongoing studies are directed at relating the effects of intravenous nicotine on rCMRglc in smokers and nonsmokers with simultaneous effects on mood and feeling state. It is anticipated that these studies will help lead to a better understanding of brain mechanisms that which underlie the behavioral effects of nicotine and support the smoking behavior.

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INCREASED SENSITIVITY OF BRAIN MUSCARINIC RECEPTOR TO ITS AGONISTS AS A RESULT OF REPEATED NICOTINE PRETREATMENT IN RATS, MICE AND RABBITS

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It has been shown by our lab[1,2] that in rats anticholinesterase(antiChE) physostigmine or soman-induced EEG seizures are characterised by initial tonic seizures of short duration and subsequent long-lasting clonic ones. The brain nAChR and mAChR were suggested to be responsible for the tonic and clonic seizures respectively. Brain nAChR can be desensitised by nicotine or antiChE.

This work is attempted to investigate the changes of brain mAChR sensitivity to its agonists after the desensitisation of brain nAChR.

In gallamine-immobilised and artificially ventilated rats nicotine 1 mg/kg iv produced EEG high frequency tonic discharges lasted about 30 sec. Nicotine 0.5-0.75 mg/kgiv induced EEG desynchronisation, but 10 min later nicotine 2.0-5.0 mg/kg iv could induce only transient EEG desynchronisation. 10 min after that additional much larger dose of nicotine 5.0-10.0 mg/kg iv produced neither EEG desynchronisation nor seizures. The desensitisation of brain nAChR resulted. 10 min after two injection of nicotine(0.75 and 2.0 mg/kg iv with 10 min interval) the threshold doses of M-agonists pilocarpine, arecoline and antiChE physostigmine, soman for producing EEG seizures decreased from 380,150,30 and 0.12 to 250,50,20 and 0.07 mg/kg iv respectively. These decreases could be prevented by N-antagonist mecamylamine 0.5 mg/kg iv. It was found that the threshold doses of GABAergic antagonists pentylenetetrazol and picrotoxin as well as glycine receptor antagonist strychnine for producing EEG seizures were not influenced by repeated nicotine pretreatment. Similar results were also obtained in mice and rabbits.

(^{3}H)QNB binding experiments showed that M-agonist arecoline 200 mg/kg ip decreased the Bmax value of rat brain mAChR from 713.3±79.4 to 572.0±72.1 pmol/mg pr. This down-regulation of mAChR could be prevented by atropine. After pretreatment of 5 injections of nicotine(0.5,1.0,1.0.2.0,2.0 mg/kg ip with 10 min interval) arecoline 50 mg/kg ip, which could not influence the Bmax value of mAChR, now did decrease the Bmax value from 713.3±79.4 to 456.0±90.1 pmol/mg pr.

In another series of (^{3}H)QNB binding experiments we studied the effect of nicotine on the binding profiles of brain mAChR to M-antagonists arecoline and oxotremorine. Computer analysis indicated that the displacement curves with shallow Hill coefficients were better fit to two binding site model. In presence of nicotine 0.1 nmol/L Ki value of mAChR high affinity site to arecoline decreased from 0.35±0.11 to 0.03± 0.01 umol/L and nicotine 1 umol/L decreased the Ki value to oxotremorine from 7.00± 3.95 to 0.48±0.44 nmol/L.

It is suggested that brain nAChR desensitisation as a result of nAChR excitation by repeated nicotine selectively increases the sensitivity of brain mAChR high affinity binding site to M-agonists.

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REGULATION OF OPIOID PEPTIDE GENE EXPRESSION BY NICOTINE

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In the present study, the effects of nicotine administration on the levels of mRNAs coding for the three opioid peptide precursors (proopiomelanocortin (POMC), proenkephalin (PENK) and prodynorphin (PDYN)) in various rat tissues were investigated.
Various modes of nicotine applications were tested: One group of rats was acutely treated with two high doses of nicotine (0.5 mg/kg) and killed one day later. These animals showed a slight decrease in the levels of POMC mRNA in the intermediate lobe of the pituitary, but no change in anterior lobe of the pituitary and in the hypothalamus. PENK mRNA levels in various brain structures (hypothalamus, striatum, hippocampus) were essentially unaffected. However, a dramatic increase in the PENK mRNA levels was found in the adrenal medulla. PDYN mRNA levels in the striatum and hippocampus remained unchanged. A marked increase, in the PDYN mRNA levels, however, was seen in the hypothalamus. This increase was associated with an increase in the levels of mRNA coding for provasopressin.
Similar changes in the opioid peptide mRNA levels were obtained when rats were chronically infused with nicotine via osmotic minipumps (4 -10 mg/kg/s.c.) for 4 days. These changes became much less pronounced after 7 days tonic nicotine infusion and disappeared completely when the rat were tonically treated with nicotine for 2 weeks. This indicates the development of tolerance to these effects of nicotine. Interestingly, the mRNA levels coding for the α_3-subunit of the neural nicotinic receptors in the hypothalamus and the adrenal medulla remained unchanged suggesting that the tolerance to nicotine is not reflected by biosynthetic alterations of this particular nicotinic receptor subunit. On the other hand the development of tolerance appears to depend on the mode of nicotine administration. Thus, chronic application of nicotine (10 mg/kg/d) in a pulsatile fashion via minipumps (3 to 4 pulses/day) for 1 weeks still resulted in dramatic increases in the levels of PENK mRNA in the adrenal medulla.

The mechanism of the induction of PENK gene expression was studied in bovine adrenal medullary chromaffin cells in culture. Incubation of the cells with nicotine (10^{-6} M) resulted in a time-dependent up to 5 fold increase in PENK mRNA levels. After an initial lag phase (with no major alterations) PENK mRNA was markedly increased between 6 to 12 hours followed by a slower, steady increase up to 48 hours. The nicotinic antagonist tubocurarine (4×10^{-7} M), the Ca^{2+}-channel blocker D 600 (10^{-5} M) and the protein synthesis inhibitor cycloheximide (10^{-5} M) prevented the rise of PENK mRNA when given within the lag phase (0 to 4 hours after nicotine). This indicates that continuous entry of Ca^{2+}-ions and de novo synthesis of protein-like transcription factors are required for the activation of PENK gene expression by nicotine.

In conclusion, our results provide evidence that the administration of nicotine into rats results in specific alterations in opioid peptide gene expression. A particularly high increase in PENK mRNA levels was found in the adrenal medulla. Tonic application of nicotine for 1 to 2 weeks resulted in the development of tolerance to these effects. This tolerance development was not associated with any alterations in the gene expression of the α_3- subunit of neural nicotinic receptors. The development of tolerance to nicotine was not evident, when the drug was chronically applied in a pulsatile fashion. As revealed by in vitro experiments with bovine chromaffin cells, the induction of PENK gene expression by nicotine involves continuous influx of Ca^{2+}-ions and de novo synthesis of protein-like transcription factors.

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The Mesolimbic Dopaminergic System as a Target for Nicotine

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Nicotine exerts diverse psychopharmacological actions both in man and in laboratory animals. The complexity of nicotine's profile of action probably reflects, in part, the widespread distribution of nicotinic receptors in the mammalian brain. Despite the large number of brain nuclei which appear to express these receptors, one neuronal pathway has received particular attention from several researchers - the mesolimbic dopaminergic system. This work, originating from different laboratories and using different experimental approaches, will be reviewed. It appears that nicotine, like the psychomotor stimulants d-amphetamine and cocaine, activates this pathway, and this action may have important behavioural consequences.

Mesolimbic dopaminergic neurons, which have their cell bodies in the ventral tegmental area of the mesencephalon, project principally to the nucleus accumbens and olfactory tubercle. All three areas have moderate to high densities of high-affinity ^{3}H-nicotine binding sites, and selective removal of dopaminergic neurons with the neurotoxin 6-hydroxydopamine leads to a marked reduction in ^{3}H-nicotine binding at the level of the cell bodies/dendrites and in the terminal regions as well. In addition, experiments using in situ hybridization histochemistry and immunohistochemistry have detected mRNA species and immunoreactivity associated with nicotinic receptors within the ventral tegmental area.

Electrophysiological experiments have confirmed that mesolimbic dopaminergic cells express nicotinic receptors and are stimulated by direct or systemic administration of nicotine. In vitro experiments show that nicotine can also act directly on dopaminergic terminals to promote the release of this neurotransmitter in the nucleus accumbens. Nicotine also enhances dopamine release from mesolimbic terminal regions in freely-moving rats, but the principal site of action is as yet unclear. Another open question is whether nicotine can stimulate mesolimbic dopamine release when the drug is given by a chronic continuous infusion rather than by acute administration.

Acute administration of nicotine can stimulate locomotor activity in rats, and this effect is more readily seen after repeated prior exposure to the drug. The locomotor stimulant effect is central in origin and reflects a stimulation of receptors. Psychomotor stimulant drugs such as d-amphetamine and cocaine appear to increase locomotor activity by enhancing dopaminergic transmission in mesolimbic terminal areas. This possibility has been examined for nicotine. In rats previously given the drug on a daily basis, nicotine produced a dose-related and stereoselective locomotor stimulant effect which was accompanied by a parallel increase in dopamine utilization in mesolimbic terminal regions. Selective depletion of mesolimbic dopaminergic terminal fields abolished the locomotor stimulant effect, indicating that nicotine-induced locomotion is not only accompanied by, but is dependent upon, activation of the mesolimbic dopaminergic system.

In summary, one important action of nicotine in the brain appears to be an activation of mesolimbic dopaminergic neurons. This action may underlie, *inter alia*, the behavioural stimulant effects of the drug.

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PRESYNAPTIC ACTIONS OF NICOTINE IN THE CNS

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Nicotine stimulates the release of neurotransmitters in the CNS. One locus of its action is directly on the nerve terminal, through presynaptic nicotinic acetylcholine receptors. The nicotinic stimulation of dopamine release from striatal nerve terminals has been particularly widely studied. Nicotine acts in a dose-dependent manner (EC_{50}=4μM) to elicit Ca^{2+}-dependent dopamine release. The pharmacological profile of this action shows that other nicotinic agonists such as cytisine and DMPP can also evoke transmitter release, and their action can be blocked by antagonists such as dihydroβerythroidine and mecamylamine; the neuromuscular nicotinic antagonist αbungarotoxin is without effect. This pharmacology favours a ganglionic type of nicotinic receptor, but nicotine-evoked dopamine release is sensitive to agents such as histrionicotoxin that block the integral ion channel of muscle nicotinic receptors, suggesting a common mechanism in the brain receptors. Molecular biological techniques have revealed several isoforms of nicotinic receptors in the CNS. The pharmacological specificity of nicotine-evoked transmitter release is consistent with the involvement of the receptor class identified by high affinity [^{3}H]nicotine binding. This correlation is also supported by the loss of such binding sites following degeneration of pathways after lesion experiments or in degenerative diseases. Subcellular fractionation expermients have shown an abundance of [^{3}H]nicotine binding sites associated with isolated nerve terminals (synaptosomes). Thus the presynaptic localisation of nicotinic receptors may constitute a major proportion of nicotine's target sites in the brain.

The high affinity binding of [^{3}H]nicotine suggests that these nicotinic receptors may rapidly desensitize, with conversion to a high affinity state. Desensitization is seen at the presynaptic receptor in the diminishing responses to successive applications of micromolar concentrations of nicotine. Desensitization is believed to underlie the well-documented increase in numbers of [^{3}H]nicotine binding sites that accompanies chronic nicotine administration. This receptor upregulation was also produced in rats by chronic infusion of the nicotinic agonist anatoxin-a. <u>In vitro</u> assessment of receptor function, by measuring nicotine-evoked dopamine release, indicated that the increased number of binding sites was accompanied by a parallel increase in receptor function. This implies that recovery from desensitization had occurred. Thus nicotine receptors in the brain may be delicately balanced between activatable and desensitized states.

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Role of the Forebrain Cholinergic Projection System in Performance in the Radial-Arm Maze in Memory-Impaired Rats.

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Impairments in performance in the radial-arm maze (involving deficits in learning, memory and/or attention) were produced in rats by one of two means: chronic administration of alcohol for 6 months, or excitotoxic lesions of the cholinergic projection nuclei to the neocortex and hippocampus. After both kinds of intervention, the animals showed profound and enduring deficits in 4 different components of memory tested in the task: reference and working spatial memory, and reference and working cue memory. Transplants of foetal cholinergic-rich brain tissue, in a cell suspension, placed into neocortex and/or hippocampus substantially reversed deficits in all 4 memory components over a time course of approximately 7-13 weeks after transplantation. Transplants of cholinergic-poor brain tissue were without effect. Both alcohol-treated and lesioned animals showed significantly reduced cortical and hippocampal levels of choline acetyltransferase (ChAT) activity. However, in rats with cholinergic-rich, but not cholinergic-poor transplants, cortical and/or hippocampal ChAT activity was elevated to control level, commensurate with site of transplant. The rats were tested prior to transplantation to determine their reactions to acute systemic administration of a range of compounds, including muscarinic and nicotinic agonists and antagonists. Both the alcohol-treated and lesioned animals showed increased sensitivity to both the beneficial effects on cognitive performance of cholinergic agonists, and the deleterious effects of cholinergic antagonists. Both muscarinic and nicotinic agents were effective. Nicotine was particularly effective in improving spatial working memory in lesioned rats. However, in lesioned rats tested after transplantation with cholinergic-rich tissue, this compound had the surprising effect of making performance in the radial-arm maze worse, although it continued to improve performance in sham-transplanted animals and in animals that had received cholinergic-poor transplants.

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Studies on the role of mesolimbic dopamine in behavioural responses to chronic nicotine.

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There is good evidence that nicotine can evoke the secretion of dopamine (DA) in the mesolimbic system of the brain and that this effect mediates the psychostimulant response to the drug and its ability to act as a reward in self-administration schedules (1,4). This presentation will summarise the results of three experiments we have performed to characterise the effects of chronic nicotine on DA secretion in the mesolimbic system and the behavioural consequences of the changes observed.

In the first series of experiments microdialysis studies were performed in freely moving rats, which had been pretreated with daily injections of saline or nicotine (0.4 mg/kg SC for 5 days), using probes located in the nucleus accumbens. On the day of the experiment the animals were left in activity boxes for 2h before being given an injection of saline or nicotine (0.4 mg/kg SC). Under these conditions both acute and subchronic nicotine increased ($P<0.01$) locomotor activity and increased the concentration of dihydroxyphenylacetic acid (DOPAC) in the dialsysate ($P<0.05$). Subchronic, but not acute, nicotine increased ($P<0.01$) the concentrations of DA and homovanillic acid (HVA) in the dialysate. The increase in locomotor activity evoked by subchronic nicotine was also greater ($P<0.05$) than that observed for rats treated acutely with the drug. In a second series of experiments the mesolimbic DA system was lesioned by injecting 6-hydroxydopamine bilaterally into the nuclei accumbens. In agreement with previous studies (1,2), the lesion suppressed the locomotor responses to nicotine (0.1 or 0.4 mg/kg SC) ($P<0.01$) and d-amphetamine (0.5 mg/kg SC) ($P<0.001$) whereas pretreatment with nicotine (0.4 mg/kg daily for 7 days) enhanced the responses to nicotine ($P<0.001$) but not amphetamine. The lesion also suppressed ($P<0.05$) the activity of rats both pretreated and tested with saline but not the activity of rats tested with saline after pretreatment with nicotine (0.4 mg/kg SC). In a third study rats treated with saline, nicotine (0.4 mg/kg SC) or d-amphetamine (0.5 mg/kg SC) were trained on a shock avoidance schedule using the procedure described Morrison (3). In agreement with the previous study, nicotine-withdrawal from rats trained with nicotine caused a significant disruption ($P<0.05$) of avoidance performance. The withdrawal of d-amphetamine from rats trained with d-amphetamine also disrupted avoidance performance ($P<0.01$) whereas its administration to nicotine-withdrawn rats attenuated the effect of withdrawal.

In summary, the results of the three studies taken together provide evidence for the hypothesis that chronic treatment with nicotine may enhance its effects on DA secretion in the mesolimbic system and that this effect of the drug may be associated both with the enhanced locomotor responses observed in rats treated chronically with the drug and the development of nicotine dependence.

These studies were supported by grants from the MRC (UK) and Wellcome Trust.

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Regulation of Intravenous Nicotine Self-Administration

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We have obtained reliable nicotine self-administration in rats using a limited-access FR5 schedule. Self-administration behavior in these studies is dose-dependent and produces nicotine intake as high as 0.5 mg/kg/hour. However, the dose-dependency of nicotine self-administration is different from that of other drugs. With nicotine, compensatory increases in responding typically do not occur as the unit dose of the drug is decreased. There is, however, a decrease in responding at high doses. Therefore, analogous to cigarette smoking by humans, regulation of nicotine intake in this IV self-administration paradigm is most apparent at the upper end of the intake curve.

This paradigm has been used to investigate some of the neurochemical substrates in the regulation of nicotine reinforcement, particularly opiate and dopaminergic systems. Based upon data from experiments in which animals were pre-treated with the antagonist naltrexone before self-administration sessions, we have concluded that an endogenous opiate system is not involved in nicotine reinforcement. However, as detailed below, evidence suggests that there is a dopaminergic substrate in nicotine self-administration.

In one study, dopamine antagonists were administered acutely prior to self-administration sessions. The effects of these antagonists on nicotine self-administration were compared to their effects on operant responding maintained by cocaine and by food, and locomotor activity after saline and nicotine injections. Pre-treatment with the D2 antagonist spiperone decreased nicotine self-administration dose-dependently. Spiperone did not alter the ability of the animals to perform, since nicotine-maintained responding was not reduced during the first half of the one-hour sessions, but decreased in the latter half. Locomotor activity after nicotine, but not after saline, was also reduced by spiperone treatment, whereas cocaine self-administration was increased.

Decreases in nicotine self-administration were produced by the D1 antagonist SCH23390 as well. However, SCH23390 also reduced operant responding maintained by food, and both the food and nicotine response patterns showed that general rate-decreasing effects could not be ruled out as the cause. The D1 antagonist also produced decreases in locomotor activity after either saline or nicotine, further evidence that the compound may have behavior-decreasing properties. Nonetheless, treatment with low doses of SCH23390 produced small compensatory increases in cocaine-maintained behavior.

Overall, therefore, these data demonstrate that pharmacological manipulation of dopamine receptors, particularly those of the D2 subtype, can alter nicotine self-administration and locomotor activity. However, the effects on self-administration are not analogous to the effect of dopamine antagonists on self-administration of cocaine. The difference may be due to differences in the regulation of self-administration of the two drugs, or may indicate that the effect on nicotine self-administration is not due to a reduction in reinforcement per se.

In another experiment, we examined whether the mesolimbic dopamine projection is involved in nicotine self-administration. After bilateral lesions of the nucleus accumbens with 6-hydroxydopamine, nicotine self-administration was reduced by approximately 75% compared to sham-operated animals. Post-mortem analysis of brain tissue showed that the lesion had produced a 93% depletion of dopamine in the accumbens and a 25% depletion in the caudate-putamen. The dopaminergic projection to the nucleus accumbens may therefore play an important role in regulating nicotine self-administration.

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NICOTINE-SEEKING BEHAVIOR IN RHESUS MONKEYS

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Nicotine is known to have psychic dependence potential. In animal experiments, this is demonstrated as the reinforcing effect of nicotine in self-administration of the drug. Several aspects of nicotine-seeking behavior in rhesus monkeys will be discussed in this paper.

1. Characteristics of nicotine-seeking behaviors

In self-administration experiments of nicotine in monkeys, when the monkeys were given free access to nicotine intravenously by pressing a lever switch, their nicotine-seeking behavior appeared mostly during the daytime and each monkey's daily ingested dose was relatively stable (1~4 mg/kg/day). On the other hand, when nicotine was made available in the form of self-administered cigarette smoke, the nicotine-seeking behavior was again observed mostly during the daytime and the serum nicotine levels of some monkeys reached up to 40 ng/ml, but their daily numbers of puffs were relatively erratic (1,000~3,000 puffs/day). It is unclear why nicotine-seeking behavior is stable by the intravenous route yet unstable when ingested by smoking, but some aversive stimuli may be responsibe for the latter.

2. Intensity of nicotine-seeking behavior

As a method to measure the intensity of drug-seeking behavior, a progressive ratio schedule is usually used in self-administration of a drug; the number of lever presses requisite for a drug administration is progressively increased at each drug intake until reaching a breaking point at which the lever pressing is abandoned. Under this schedule with nicotine 0.25 mg/kg/infusion, some monkeys reached final ratios of 2,000~3,000:1. These values can be regarded to be significantly high, although much lower than those for opiates or cocaine. In the case of opiates such as morphine or codeine, it is well known that pretreatment with the drugs prior to the progressive ratio test markedly enhances the drug-seeking behavior, but nicotine pretreatment at hourly doses of 0.25 mg/kg for 4 weeks was not found to enhance the nicotine-seeking behavior.

3. Induction of nicotine-seeking behavior and serum concentration

Intravenous self-administration experiments were conducted at 3 unit doses of nicotine (30, 120, and 240 μg/kg/infusion) in 4 monkeys each in random order. During each 10- to 14-day self-administration period at a fixed unit dose, the serum concentration of nicotine was determined 4 times in each monkey immediately following a lever press and before infusion to find the concentration at which the drug-seeking behavior was induced. As a result, these concentration levels (17.6, 37.3, and 60.1 ng/ml, respectively) were found to be considerably different depending on the daily ingestion level of nicotine (0.9, 2.3, and 4.0 mg/kg/day on the average, respectively). Thus it can be said that the higher the daily ingestion level of nicotine, the higher the serum concentration at which nicotine is sought.

4. Nicotine-seeking behavior and nicotine discrimination behavior

Since drug-seeking behavior is generally believed to be initiated and maintained as a result of reinforcing subjective effects, the dose relationship between the reinforcing effect and discriminating effect of nicotine was examined by observing the intravenous threshold doses in self-administration and drug discrimination experiments in 2 monkeys each. As a result, the threshold unit doses were 15 and 30 μg/kg in the former and 25 μg/kg for both in the latter. This result may indicate that nicotine-seeking behavior is developed as a consequence of its

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Genetic Regulation of Behavioral and Physiological Sensitivity to Nicotine

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Numerous studies have demonstrated that the administration of an acute dose of nicotine results in alterations in a broad spectrum of behavioral and physiological measures. At least for some measures, both stimulant and depressant effects may be observed. This complexity has frustrated investigations that have been directed towards understanding the neurochemical measures that regulate nicotine response.

Our research group has chosen to use genetic strategies to attempt to broaden our understanding of those mechanisms that regulate the sensitivity of mice to nicotine. These strategies have involved an analysis of the relative sensitivities of a large number (19) of inbred mouse strains to nicotine. Most of our studies have assessed the effects elicited by injection with low doses of nicotine on rate of respiration, locomotor activities measured in either a Y-maze or an open-field arena, acoustic startle response, heart rate and body temperature. We have also studied nicotine-induced seizures. In all cases, differences among inbred mouse strains have been detected. However, a mouse strain that was sensitive to one effect of nicotine or measured by an ED_{50} value was not necessarily sensitive to all other nicotine effects. Correlational and principal component analyses indicate that a minimum of two major families of responses to nicotine exist. One factor loads heavily on nicotine effects on locomotor activities and body temperature and another factor loads heavily on seizures. Nicotine effects on the other measures seem to be regulated by both of the major factors. The numbers and affinities of L-[^{3}H]nicotine and α-[^{125}I] bungarotoxin were also measured in eight brain regions. Mouse strains differ in the number, but not the affinities, of both of these binding sites. The two binding sites segregated independently of one another suggesting separate genetic control. Correlation between sensitivities to nicotine and nicotinic receptor numbers were calculated and significant correlations (r = approximately 0.6) were found between nicotine effects on locomotor activities and body temperature and [^{3}H] nicotine binding on one hand and nicotine-induced seizures and α-[^{125}I]bungarotoxin binding on the other hand.

More recent studies have focused on corticosteroid regulation of sensitivity to nicotine and on nicotinic receptor binding. Only a few strains have been analyzed to date but the results available clearly demonstrate that inbred mouse strains differ in the effects of adrenalectomy and corticosterone on sensitivity to nicotine. Data currently on hand indicate that corticosterone inhibits binding to brain nicotinic receptors in several mouse strains. Whether this inhibition varies between mouse strains is currently under investigation.

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PUTATIVE NEUROELECTRIC CORRELATES OF SMOKING BEHAVIOR

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Since the various smoking-related changes in mood states and behavior are presumably mediated by alterations in neuronal activity, considerable research effort has been devoted to uncovering possible neurophysiological correlates of acute cigarette smoking. The scalp-recorded electroencephalogram (EEG) provides an ongoing record of neuroelectric activity of the brain, and although admittedly it is a gross measure of brain function, lacking anatomic depth and specificity, it provides the best and most direct objective measure currently available for continuous assessment of the functional state of the CNS.

Although the usefulness of EEG is obviously enhanced when it is supplemented with concurrent subjective/behavioral measures, it is suggested here that the characterization of EEG profiles of smoking behavior may, in their own right, provide insight into the psychological/motivational states underlying tobacco use. The purpose of this paper is to present the results of three brain electric studies which attempted to:

(1) quantify the acute dynamic and static EEG profile changes as assessed during and immediately following the smoking of a single cigarette;

(2) examine the similarities/differences of acute EEG profiles derived from the smoking of low and medium tar-nicotine (T/N) yield cigarettes and

(3) assess the scalp distribution of EEG changes induced by the smoking of cigarettes with varying T/N yield.

The results of these studies indicate that: (1) smoking exerts a psycho-stimulant EEG profile which is charaterized not by desynchronization, i.e. alpha reduction, but by alpha enhancement and is evident by the first 4 to 5 puffs; (2) smoking low T/N yield cigarettes exerts a similar but less intense EEG profile change as observed with medium T/N yield cigarettes and that (3) the smoking of cigarettes with increasing T/N yields results in a progressive posterior-to-anterior spreading of EEG effects across the scalp. The significance of these neuroelectric profiles are discussed in relation to theories of smoking motivation.

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COMPARATIVE PSYCHOPHARMACOLOGY: PUTTING NICOTINE IN ITS PLACE

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Psychopharmacologists assume that the psychotropic properties of a substance will be directly related to the changes in behaviour and psychological function following administration of appropriate doses of the drug. However, to look at the effects of a psychoactive substance per se, without the benefit of comparative data from other psychotropics, creates problems in placing a drug in its proper position relative to other psychoactive substances. This paper reports the results of a meta-analysis of the effects of a range of psychoactive substances (including nicotine, caffeine and alcohol) on valid and reliable psychometric measures.

The results show that the overall effects of nicotine in improving all aspects of cognitive and psychomotor function are greater than those of placebo, but not as great as the effects produced by methylphenidate, amphetamine and pemoline.

Nicotine is also significantly and distinctly separated by its profile of psychopharmacological activity from substances such as morphine, codeine, minor tranquillisers and some anti-depressants and neuroleptics. Nicotine is closer in its profile of behavioural action to caffeine and some agents used as cognitive enhancers.

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Relationships between Photic Driving, Nicotine and Memory

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In the present study an attempt is made to converge and harmonize three sets of observations. The first focuses on the reported enhancement effect of nicotine on initial learning, immediate memory and on the consolidation process underlying long-term memory. It is well established that nicotine excites electrocortical arousal, indicated by EEG desynchronisation, decreased EEG amplitude and increase in dominant alpha frequency. These effects are attributed to a secondary release of acetylcholine following stimulation of nicotinic receptors in the mesencephalic reticular formation. Further evidence suggests that the cellular layers of the hippocampus, an organ implicated in attentional and memory processes, are a specific target for nicotine. The second set relates to evidence published in the Soviet literature relating certain parameters of the Photic Driving (PD) response to efficiency of incidental and intentional memory, both short- and long-term. The third set refers to studies investigating the effect of nicotine on PD efficiency, which have thrown up conflicting results, and studies reporting that nicotine improves critical flicker fusion (CFF), a measure which correlates highly with PD efficiency. The experiment involved the measurement, using a repeated measures design, of PD power at 14 frequencies across 16 EEG sites, and measurement of immediate recall of passages of discursive prose (Rivermead Test), in 40 subjects under 4 nicotine conditions. Results showed significant relationships between high frequency PD power and memory, between nicotine dose and PD power, and between nicotine dose and efficiency of recall, all in the predicted directions. Implications of these findings are discussed.

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Effects of nicotine on serial memory search

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Two studies are described in which the effects of smoking a nicotine versus non-nicotine cigarette on performance on Sternberg's serial memory search task was compared. The memory search task involves subjects being shown a short list of digits and then being presented with digits one at a time and being asked to indicate as quickly as possible in each case whether this "probe" was a member of the "positive set" which they had previously seen. As a positive set size increases there is a linear increase in response times. In the version of the task used for the present studies positive set sizes of 2 and 5 were used. In the first study, a group of 10 occasional smokers performed the memory search task. Occasional smoking was defined as less than 5 cigarettes per day on average and at least one day per week without smoking at all. Having been tested on the task the subjects smoked either a nicotine or non-nicotine cigarette. After this, they performed the search task again. Then they smoked either a non-nicotine or nicotine cigarette (whichever one they had not smoked before) and their memory search performance was tested again. The results indicated that the search rate (the estimated time taken to match the probe digits with their stored representation of each item in positive set) was significantly faster after the nicotine cigarette than the non-nicotine cigarette.

A second study was carried out to confirm and extend this finding. A group of 14 occasional smokers and 15 regular smokers underwent the same testing protocol as was used in the first study. In both groups the tests were carried out before and after a period of 24 hours' abstinence from cigarettes. The occasional smokers experienced no significant withdrawal symptomatology and scored low on the dependence subscale of Russell's smoking motivation questionnaire. The results indicated as before that the search rate was faster after the nicotine versus non-nicotine cigarette and that there was no significant difference attributable to withdrawal state or between regular and occasional smokers.

The results indicate that smoking a cigarette containing nicotine can speed processing involving short-term memory and that this effect does not appear to be subject to significant chronic or acute tolerance under normal smoking regimes.

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Nicotine and cardiovascular disease

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Cigarette smoking is a major risk factor for coronary and peripheral vascular disease. This paper addresses the importance of nicotine per se in causation of heart disease. In people with coronary artery disease, nicotine, by increasing the work and oxygen demand of the heart, can induce ischemia that may contribute to reduced exercise capacity and/or impaired myocardial contractility. Coronary spasm and enhanced thrombosis are believed to contribute to smoking-related myocardial infarction. Nicotine may contribute to coronary spasm by sympathomimetic actions or inhibition of local release of prostacyclin. The role of nicotine in causing hypercoagulability has not been established. Nicotine could promote atherosclerotic vascular disease by actions on lipid metabolism and coagulation, by hemodynamic effects and/or by causing endothelial injury. Nicotine feeding has been shown to increase LDL cholesterol in some animal studies, but such effects have not been confirmed in most studies of humans chewing nicotine gum. Nicotine produces structural damage to aortic endothelial cells in animals, but the significance of this effect to human disease is unclear. Arrhythmias could contribute to sudden death in smokers. Cigarette smoking does not increase the prevalence of ventricular ectopy in people with stable ischemic heart disease. However, nicotine facilitates AV nodal conduction and may render arrhythmias that occur due to acute ischemia more life-threatening. In summary, nicotine may contribute to tobacco-related cardiovascular disease, but direct causation has not been determind. Short-term nicotine therapy, such as nicotine replacement therapy as an adjunct to smoking cessation therapy, poses little cardiovascular risk to healthy individuals.

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Nicotine and smoking in patients with ulcerative colitis

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The chance observation in 1982 that very few patients with ulcerative colitis were current cigarette smokers[1] has encouraged much research into this association and the publication of a number of anecdotal reports. The new evidence has substantiated and expanded the original findings and has provided a new approach to investigate the pathogenesis of ulcerative colitis.

The original observation came from an investigation into the occurrence of lung disorders in patients with inflammatory bowel disease. Despite a high incidence of pulmonary abnormalities, only 11% of patients with ulcerative colitis were current cigarette smokers. In contrast, 36% of patients with Crohn's disease smoked, a figure similar to the national average.

Shortly after, Harries et al.[2] carried out a specific questionnaire survey and found that only 8% of ulcerative colitis patients were current cigarette smokers as compared with 42% of Crohn's disease patients and 44% of age- and sex-matched controls.

Following these reports, supporting anecdotal evidence linking non-smoking with ulcerative colitis was published. De Castella[3] described the case history of a 33-year old woman smoker who abruptly stopped and shortly afterwards developed the classic symptoms of ulcerative colitis.

Initially, nicotine was proposed as the active constituent of cigarette smoke responsible for this effect. This followed a report by Roberts & Diggle[4] of an individual for whom remission of symptoms could be induced equally by resuming cigarette smoking or by 16mg of nicotine per day delivered from nicotine chewing gum.

Since these original observations, international interest has been intense. There is no doubt that the risk of ulcerative colitis is very much lower among current cigarette smokers than among those who have never smoked, the estimated relative risk (RR) being 0.25 ($p<0.001$). There is also no doubt that such a reduction in risk is not evident in ex-smokers[5].

Although many theories have been advanced, little experimental evidence has been forthcoming to explain these findings. Based on the knowledge that colonic mucus is known to be quantitively and qualitatively abnormal in ulcerative colitis, and that cigarette smoking alters respiratory mucus by systemic as well as local effects, we have examined colonic mucus production in vitro in patients with ulcerative colitis. The results show that patients with ulcerative colitis have significantly lower colonic mucus production compared with controls. When smoking habits were taken into account, the majority of patients who were non-smokers had reduced mucus production compared with non-smoking controls. Those patients who did smoke had a level of colonic mucus production that was equivalent

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to smoking controls. Smoking appeared to have no effect on colonic mucus production in controls since both smokers and non-smokers had the same level of mucus production[6,7]. It would appear from these results that smoking does not affect mucus production under normal circumstances, but in a situation where mucus production is abnormal, as in ulcerative colitis, then smoking appears to have a restorative effect on mucus production. In vitro neither nicotine nor its major breakdown products had any effects on colonic mucus synthesis.

These studies suggest that smoking has profound influence on colonic mucus production, rendering patients' physical colonic mucosal defences relatively suppressed. These changes may well have relevance to the pathogenesis of inflammatory bowel disease.

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Smoking and Parkinson's Disease

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In a case control study involving 383 patients with Parkinson's Disease a negative association with smoking was demonstrated. The relative risk of developing Parkinson's Disease was approximately halved in current smokers and a history of smoking up to 20 years earlier was shown to reduce the relative risk of developing the disease.

The negative association between Parkinson's Disease and smoking is independent of other associated factors.

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NICOTINE AND HUMAN COGNITION

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The profound reduction in nicotine receptors in the neocortex and hippocampus of patients dying with dementia of the Alzheimer type (DAT) suggests that stimulation of the remaining receptors with systemic nicotine might help in the development of an alternative approach to cholinergic treatment of this condition, when early in the course. The results of a preliminary study of subcutaneous nicotine in groups of young normals, elderly normals and patients with DAT (all n=7) are described. Tests of rapid visual information processing (non-spatial working memory), short term spatial working memory and cortical arousal (critical flicker fusion test (CFF)) were modified to allow the baseline performance of the DAT patients to approximate that of normal subjects. Nicotine (0.4, 0.6, and 0.8 mg) produced dose-dependent improvements in the DAT patients in the accuracy and speed of responding in the rapid information processing paradigm, reduced the threshold for CFF, but had no beneficial effect on the test of spatial short term memory. These preliminary observations are extended in two ways: by studying the effects of acute nicotine on larger populations of subjects, including equivalent numbers of smokers and non-smokers; and by investigating the effects of chronic nicotine, administered in the form of chewing gum, on similar measures, but also including an evaluation of effects on indices of everyday living.

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Nicotinic receptors in brains of healthy individuals and Alzheimer patients as visualized in vivo by positron emission tomography and ^{11}C-methyl nicotine.

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Alzheimer´s disease is a progressive neurodegenerative disorder with global deterioration of cognitive function. Among the afflicted transmitter systems in brain the cholinergic system is the one that correlates best with cognitive function. Several postmortem studies now confirm the marked reduction in cortical nicotinic receptors density in Alzheimer´s disease. The decrease in nicotinic receptors has been confined to the biological active (S) enantiomer of nicotine. The results are based on material obtained at autopsy or by biopsy and have to be confirmed in vivo.

Attempts to visualize nicotinic receptors in vivo using ^{11}C-nicotine and positron emission tomography (PET) have recently been made in monkey and man. The aim of the present study was to assess by PET the use of (-) (S) nicotine labelled with ^{11}C as well as the enantiomer of nicotine, with lower receptor affinity, the (+) (R) form, as markers for nicotinic receptors and/ or unspecific disposition of ^{11}C-nicotine in brain of healthy volunteers and Alzheimer patients.

The nicotinic enantiomers (-)(S) and (+)(R) ^{11}C-nicotine was injected to six patients with the diagnosis Alzheimer´s disease (agen range 63-74,duration of disease 1-10 years) and six age matched healty volunteers.None of the subjects were smokers. The radioactivity from (+) and (-) ^{11}C-nicotine was rapidly distributed from arterial blood after i.v. injection. The radioactivity peaked in brain within 2-5 minutes and was then followed during 30 mins. The uptake of ^{11}C-nicotine in healthy volunteers was high in brain areas as the thalamus, caudate nucleus, putamen, frontal and temporal cortex, intermediate in the occipital cortrex , cerebellum and low in white matter . The (+)(R) and (-)(S) ^{11}C-nicotine enantiomers showed a similar uptake and pattern of distribution in control brains .

In Alzheimer brains on the other hand, a lower uptake of (+)(R) ^{11}C-nicotine was observed in comparison to (-)(S) ^{11}C-nicotine . The uptake of (-) and especially (+)^{11}C-nicotine to cortical areas was in general lower in Alzheimer patients compared to controls. ^{11}C-butanol, a marker for cerebral blood flow, had a different time course in the brain in comparison with ^{11}C-nicotine and clearly support the assumption that ^{11}C-nicotine has a specific binding profile in brain and that changes in uptake and time course of ^{11}C-nicotine reflect changes in specific binding.

In conclusion ,the pattern of distribution of ^{11}C-nicotine in brain resembles the distribution of nicotinic binding sites. A significant difference in uptake of (-) and (+) ^{11}C-nicotine to the brain is observed in Alzheimer patients compared to healthy volunteers. Further PET studies in man will give valuable information concerning the role and physiological function of the nicotinic receptors subtypes in brain

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1

EXTRAHEPATIC COMPARED WITH HEPATIC METABOLISM OF NICOTINE IN THE RAT

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Pulmonary and hepatic metabolism of ^{14}C-labelled nicotine and cotinine was studied in isolated perfused lung and liver of phenobarbital (PB)-pretreated and uninduced rats. The kinetic parameters of nicotine were also investigated in conscious rats receiving nicotine (0.3 mg/kg) either intravenously or orally in order to quantify hepatic first-pass extraction of nicotine in vivo.

The kinetic parameters of the elimination of nicotine from the perfusion circuit demonstrated a relatively high elimination rate in rat lung approaching the capacity of liver when both organs were not induced. The concentration-time profiles of the metabolite cotinine in the perfusate were almost identical in perfused lung and liver. In the untreated conscious rat 89 % of the oral dose reached the circulation as unchanged nicotine indicating a low hepatic extraction ratio of about 10 %. - The turnover of the metabolite cotinine was studied in isolated perfused organs using cotinine as the substrate. In the uninduced liver the elimination half-life was 180 $\pm$ 27 min and the clearance was 0.5 ml/min corresponding to a low value of 4 % for the first-pass extraction. The isolated rat lung exhibits much less activity to metabolize cotinine. Thus a secondary metabolic pathway of cotinine predominantly occurs within the liver.

Phenobarbital treatment had different effects on nicotine metabolism in lung and liver. The clearance of nicotine was induced 2-fold in lung. The pattern of nicotine metabolites in the perfusate, however, was not affected by PB-induction. The pulmonary capacity to metabolize cotinine was not different from uninduced controls. Hepatic nicotine elimination was induced 8-fold by PB, as shown previously for the isolated rat liver. A marked increase of the hepatic first-pass extraction of nicotine was now confirmed for the in vivo situation in which the oral availability of unchanged nicotine dropped to 1.4 %. Yet, despite of the marked induction of the hepatic clearance, the total clearance of i.v. nicotine was only increased 2-fold in the conscious rat. This indicates that an important part of the systemic clearance of nicotine is mediated by extrahepatic organs.

In the perfused liver the elimination parameters of cotinine used as the substrate were increased approximately 10-fold after PB. This is in accordance with the very small amount of cotinine appearing in the plasma after i.v. administration of nicotine to the conscious rat. The pattern of urinary metabolites of nicotine strongly correlated with the induction state. It is concluded that the metabolism of cotinine, which predominantly occurs in the liver, may be a suitable indicator of the PB-inducible cytochrome P-450 isoenzyme(s).

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2

CONTRIBUTION OF TRANS 3'-HYDROXYCOTININE AND GLUCURONIDE CONJUGATES OF NICOTINE METABOLITES TO THE MEASUREMENT OF COTININE BY RIA.

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Interlaboratory studies have shown that cotinine measured in urine by radioimmunoassay (RIA) generally gives results which are higher than those obtained by gas chromatography (GC) methods of analysis. However, cotinine standards added to urine results in complete recovery by both methods. Recent advances in nicotine metabolism have determined trans 3'-hydroxycotinine to be the major nicotine metabolite present in urine and have further identified glucuronide conjugates of nicotine, cotinine and 3'-hydroxycotinine. These newly found metabolites are not readily quantitated by GC methods of analysis and their contribution to immunoassay methods have not been determined. We hypothesized that the higher RIA results observed in comparison studies could arise from cross-reactivity with one or more of these metabolites. To test this hypothesis, we conducted a series of studies to characterize our antibody and to evaluate a spectrum of nicotine metabolites in the urine of smokers.

Authentic trans 3'-hydroxycotinine was added to urine obtained from non-smokers. Within the range of 1-300 ng/ml of added hydroxy-cotinine, the RIA assay measured approximately 35% of this nicotine metabolite. Urine samples obtained from smokers were then incubated with ß-glucuronidase for 6 hours at 37°C and the RIA cotinine levels were compared with those obtained on untreated samples from the same smokers. Results revealed no difference in metabolite detection between the two samples from each smoker. This suggests that glucuronide conjugates, if present, are detected by the RIA assay method.

Extralut 3 columns were used to extract ß-glucuronidase treated and untreated urine samples. With untreated urine, over 60% of the assayable material was retained by the column. However, when samples were incubated with ß-glucuronidase prior to column extraction, only 30% of the RIA reactive components were retained. Finally, when an inhibitor of ß-glucuronidase was added to the incubation mixture, the ß-glucuronidase effect was eliminated. These results suggest that in addition to holding back 3'-hydroxycotinine, glucuronide conjugates are retained by Extralut columns.

Overall, these results confirm our hypothesis and demonstrate that the RIA assay for urine cotinine provides measurement of free and conjugated cotinine as well as partial reactivity to 3'-hydroxy-cotinine. Experiments are being conducted to quantitate levels of individual metabolites and to explore inter-individual variation in conjugation of nicotine metabolites in urine.

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3 DETECTION OF NORNICOTINE IN HUMAN URINE AFTER THE INFUSION OF NICOTINE

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Oxidative dealkylation is a major pathway in drug metabolism. Nornicotine would be the product of nicotine by this route.

Nornicotine was found in smokers urine by McKennis, Schwartz, and Bowman (1964), and after intraarterial application of nicotine, 8.9% of the administered dose were found in the urine of rats as nornicotine by Kyerematen et al. (1987). Nornicotine was also found in recent smoker studies by Neurath et al. (1988), but the nornicotine was not in excess of 1.9%, 1.7%, and 1.4% respectively of the excreted sum of the total nicotine metabolites.

As nornicotine occurs in minor and varying concentrations in most of the tobaccos, its occurrence in the urine of smokers does not prove its formation in the human metabolism: It may be directly absorbed by the smoker and pass unchanged to the urine.

Direct evidence of its metabolic formation can best be achieved from experiments under direct application of nicotine to humans.

Therefore, the nornicotine formation after intravenous infusion of nicotine was studied in the urinary excretion of six male regularly smoking volunteers after refraining from smoking.

Nornicotine has been found in the urine presenting evidence for the existence of this metabolic pathway. But only minor amounts of this metabolite are formed - less than 1% of the administered dose of nicotine possibly indicating rapid further metabolism.

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4 Estimation of Nicotine and Tobacco Specific Nitrosamines (TSNA) in Saliva After Nicotine Infusion and After Smoking

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The contributionon of salivary glands to the excretion of nicotine and TSNA which may derive from nicotine was investigated in two studies. In the first study, four healthy male cigarette smokers received three nicotine infusions (2 $\mu g \cdot min^{-1} \cdot kg^{-1}$ for 10 min each) with an interval of 50 min between infusion 1 and 2, and an interval of 15 min between infusion 2 and 3. Nicotine and its main metabolite cotinine were measured in serum and in saliva from the parotid gland, the submandibular gland and in mixed saliva. In the second study, four healthy male cigarette smokers received one nicotine infusion as in study 1. After one hour they were asked to smoke four cigarettes within 50 minutes and a further four cigarettes two hours later. Nicotine and cotinine were monitored in serum and in pooled saliva from the parotid gland over the whole time. Saliva specimens were sampled to determine tobacco-specific nitrosamines.

Ten to twenty minutes after the beginning of the nicotine infusion nicotine concentration was about 20 times higher in saliva from the submandibular gland and about 60 times higher in saliva from the parotid gland as compared to serum levels. Intermediate values were obtained in mixed saliva. After smoking nicotine values in pooled saliva from the parotid gland were about 10 times higher than those in serum. Nicotine concentration in saliva reached peak values between 600 and 2000 ng/ml after infusion and mean values in the pooled samples between 150 and 500 ng/ml after smoking. Cotinine values in saliva are slightly higher than those in serum, but the differences are marginal. TSNA could neither be detected after nicotine infusion nor after smoking.

The increase in salivary nicotine is due to the fact that saliva is more acidic than is serum. As in the renal handling process, nicotine is rapidly protonated in the saliva and the salt is not able to cross the membrane back to blood. A comparable increase in cotinine and TSNA in saliva should not be expected since these substances are less alkaline than nicotine.

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5 Pharmacokinetics of nicotine after intranasal administration.

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Administration of nicotine in different formulations has been tried as an aid to stop smoking. As nicotine is exposed to a large first-pass metabolism and has a low oral bioavailability, the substance is a good candidate for non-oral routes of administration. Initially we performed experimental studies in rats showing a high bioavailability of nicotine following nasal administrations at different pH.

In 8 healthy subjects we then investigated the absolute bioavailability of 1 mg nicotine applied as drops on the nasal septum, nasal conchae and as a nasal spray. The study was designed as a 4-way cross-over study and after each treatment, the plasma concentrations of nicotine was followed for 6 hours post-administration. Nicotine was assayed by a capillary gas chromatography method. Furthermore, in some of the subjects the concentration of both nicotine and cotinine was followed for an extended period after administration of intravenous nicotine and after the nasal spray formulation.

As compared to an intravenous infusion of nicotine, the extent of bioavailability was between 55 and 70% for the nasal administration. The rate of absorption was fast; maximum concentrations were reached after about 10 minutes. No significant difference in rate or extent of absorption between the different nasal administrations could be seen.

In conclusion, as a nasal formulation nicotine shows a high extent of bioavailability. The absorption is rapid and reliable independent of the pattern of nasal deposition.

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6 Nicotine application with an oral capsule

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An elastic gelatin capsule has been developed which releases 4 mg of nicotine in 0.8 ml of an alkaline solutin (pH 10) into the mouth after crunching the capsule's wall. In a pilot study with 5 male smokers and 5 male nonsmokers, nicotine absorption after administration of one capsule was studied by measuring the alkaloid and its main metabolite, cotinine, in plasma and urine. Cardiovascular responses were recorded before, during and after administration of a verum and a placebo capsule. In addition, the subjects were enquired how they felt after administration of the capsule. The peak nicotine concentration in plasma was reached 10 min after application of the nicotine capsule and ranged from 4 to 10 ng/ml. In those subjects who were advised to swallow the contents of the capsule instead of spitting it out after 4 min, a second nicotine peak was observed 40 to 120 min after administration. In most of the subjects nicotine uptake through the capsule was accompanied by an increase in heart rate and systolic blood pressure and a decrease in skin temperature. Subjective response to and tolerance of the nicotine capsule varied from subject to subject. There was no significant difference between smokers and nonsmokers in any of the variables measured.

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7 TOXICOKINETICS OF NICOTINE-DERIVED NITROSAMINES IN THE RAT

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The two tobacco specific nitrosamines NNK (4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone) and NNN (nitrosonornicotine) are important toxic ingredients of tobacco and tobacco smoke. They are formed from nicotine during curing and fermentation of tobacco leaves by nitrosation. Hereby, the nitrate concentration plays a predominant role for their formation (Fischer et al., Carcinogenesis 10, 1989). Relatively high concentrations have been found in tobacco (up to 8850 p.p.b. NNN and 1400 ppb NNK) and in tobacco smoke (up to 625 ng/cig NNN and up to 432 ng/cig NNK) by Fischer et al. (Carcinogenesis 10, 1989a; 1989b). Furthermore NNN and NNK are potent carcinogens which may induce tumors locally or systemically. Accordingly, NNN has been found to induce tumors of the nasal cavity and oesophagus of rats after s.c. and oral application, whereas nasal cavity, lung, liver and pancreas tumors were induced by NNK (rev. Hecht & Hoffman, Cancer Surveys 8, 1989). Therefore these nitrosamines could well be responsible also for the human malignant tumors which occur locally in respiratory and upper digestive tract, or systemically in bladder, renal pelvis and pancreas (IARC Monograph, Vol 38) following uptake of tobacco and tobacco smoke related products.

It was the aim of this study to elucidate in which manner the active metabolites of NNN and NNK contribute to the systemic carcinogenic properties of tobacco related products. The experimental approach was to assess toxic and genotoxic effects by N-nitrosodimethylamine (NDMA), NNK and NNN in different organs of the rat. For this, induced DNA single strand breaks (SSB) were first determined in hepatocytes *in vitro* to comparatively establish the genotoxic potential of the compounds. Furthermore SSB were assessed in isolated cells of different organs following single *p.o.* application and 1 h exposure to the test chemicals. Oral application was chosen as the first exposure route, since it is relevant for the chewing and snuff dipping use of tobacco. So far, with the alkaline elution technique of Kohn et al. (Biochemistry 15, 1976) it has been found that all three compounds are genotoxic *in vitro* (NDMA = NNK> NNN) within the concentration range of 6.25 - 25 umoles/ml/10^6 hepatocytes.

Ex vivo, NDMA is a potent inducer of DNA SSB in liver (>0.1 mg/kg), lung, kidney and lymphocytes (>2 mg/kg). No genotoxicity was observed in testes or thymus. NNK induces SSB at 35 and 50 mg/kg in the liver and lower doses are being assessed. 25-100 mg /kg NNN was not genotoxic in the liver. This dose range is only 2.5-10% of its reported LD_{50}, but is equitoxic to the very genotoxic dose range of 1-4 mg/kg NDMA. We are presently evaluating NNK and NNN in additional organs (e.g. oesophagus) to identify susceptible target tissues. Since this cell yield is too low to perform the alkaline elution, genotoxic effects are detected by other techniques. Accordingly, first results with a single cell analysis of DNA damage by *in situ* nick translation (Anai et al., Cancer Letters 40, 1988) will be presented. This type of approach in toxicokinetics may aid not only in elucidating the role of individual compounds of the complex carcinogenic mixtures tobacco and tobacco smoke, but may also serve as a useful modell system for studying specific mechanisms.

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8 NO PYROSYNTHESIS OF NNN AND NNK FROM NICOTINE

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N'-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are powerful and organspecific carcinogens, which have been found in tobacco and tobacco smoke in relatively high concentrations. NNN is either formed by nitrosation of the major tobacco alkaloid nicotine under loss of a methylgroup or by nitrosation of the minor alkaloid nornicotine. NNK can only derive from nicotine by oxidative N-nitrosation. In order to reduce smokers burden with NNN and NNK it is essential to know origin and pathways of their formation. During curing and fermentation of tobacco, the tobacco alkaloids can react with nitrite, which is formed by microbial reduction of nitrate, to yield tobacco-specific nitrosamines (TSNA). Nitrate also gives rise to nitrogen oxides in smoke which are supposed to react with the tobacco alkaloids during smoking. Thus the presence of NNN and NNK in mainstream smoke was explained qby their direct transfer from tobacco into the mainstream smoke and by their formation during smoking (pyrosynthesis). The purpose of this investigation was to investigate the contribution of a possible pyrosynthesis to the TSNA burden os smokers.

Spiking of cigarettes with the TSNA precursors nitrate and nicotine prior to smoking (nitrate spiking level: 4-20 mg/cigarette, nicotine spiking level: 10 mg/cigarette) did not result in a measurable increase of NNN and NNK in mainstream smoke. After spiking cigarettes with radioactive labelled nicotine neither radioactive labelled NNN nor radioactive labelled NNK could be detected in the mainstream smoke. Furthermore the concentrations of NNN and NNK in mainstream smoke and in tobacco were determined for commercial cigarettes and the ratios between the mainstream smoke concentration and the level of preformed NNN and NNK were calculated for West German nonfilter cigarettes. These ratios were constant and did neither depend on the nicotine nor on the nitrate level of the tobacco except for NNK in dark tobacco type cigarettes.

The nitrosation potential of freshly generated mainstream smoke was investigated by addition of nornicotine and nicotine to the trapping fluids for the mainstream smoke. Nitrosation of the secondary amine nornicotine could be observed, whereas nicotine nitrosation could not be detected under these conditions. Furthermore model studies on the exposure of nicotine to nitrogen oxides have been carried out. At a total nitrogen oxide concentration of 400 ppm with varying composition of nitric oxide and nitrogen dioxide (400 ppm NO/no NO_2 - 240 ppm NO/160 ppm NO_2) only small amounts of NNN and NNK were formed.

These data indicate that nicotine cannot easily be nitrosated during smoking to form NNN and NNK. Pyrosynthesis of NNN and NNK from nicotine does not occur for standard cigarette types. The NNN and NNK burden of smokers is given by the amount of preformed NNN and NNK in tobacco.

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9 EXPOSURE TO NICOTINE-DERIVED N-NITROSO COMPOUNDS AND EVIDENCE AGAINST ENDOGENOUS FORMATION.

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The nitrosation of nicotine results in the formation of N-nitrosonornicotine (NNN) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), two potent organ-specific genotoxic carcinogens in experimental animals for the nasal cavity and lung, respectively. In addition to which, both 4-(N-nitrosomethylamino)-4-(3-pyridyl)butyric acid (iso-NNAC) and 4-(N-nitrosomethylamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) are also formed, these two compounds appear to be noncarcinogenic. Considerable evidence is available to support the carcinogenic action of the nicotine-derived nitrosamines NNN and NNK in human carcinogenesis. As a result, reductions in the exposure to both NNN and NNK from all potential exposure sources should be made.

Exposure to these two compounds may result via two potential routes: (I) Exogenous exposure to preformed nicotine-derived nitrosamines present in tobacco products, and (II) endogenous formation of these N-nitroso compounds from their chemical precursors. The endogenous formation of N-nitroso compounds (but not those derived from nicotine) has been demonstrated to occur in the oral cavity, gastrointestinal and urinary tracts.

The presence of both NNN and NNK in over 200 samples of smokeless tobaccos will be reviewed together with an estimate of the exogenous exposure to these carcinogenic compounds.

The potential endogenous nitrosation of nicotine under conditions simulating the oral cavity and stomach using artificial saliva and gastric juice was investigated using both nicotine and extracts of oral tobaccos. No evidence was found to support the endogenous nitrosation of nicotine under ***in vivo*** conditions.

It is concluded that the exposure to the nicotine-derived carcinogenic N-nitroso compounds NNN and NNK results only from the use of tobacco products containing these preformed N-nitroso compounds (exogenous exposure) and not from the ***in vivo*** nitrosation of nicotine ***per se*** (endogenous exposure).

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10 INTESTINAL FIRST PASS METABOLISM OF NNK IN THE MOUSE

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NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, belongs to the group of so-called tobacco specific nitrosamines. In animals NNK has been shown to be a strong carcinogen producing mainly tumors in the lung. Although the metabolism of NNK has been extensively studied nothing is known about its fate during absorption in the small intestine, the main site of nitrosamine exposure in smokeless tobacco users and an important one even in smokers.

Using radioactive NNK, ^{14}C-labelled at the carbonyl group, we investigated the first pass metabolism in jejunal segments of mice. Male NMRI mice, 25 - 30 g body weight, were either fed ad libitum or starved for 18 h. An additional group of mice was starved for 48 h and given acetone, 5 ml/kg, 24 h before the experiment. Jejunal segments of 8 - 10 cm length were perfused for 2 h at 37°C with Tyrode's solution containing 1 μM ^{14}C-NNK. Aliquots of perfusate (mucosal side) and absorbed fluid (mucosal side) were analysed for total radioactivity and for the presence of metabolites by radio-HPLC. The results are summarized in the following table. The values represent means ± S.E. of eight segments.

Treatment	Percentage of metabolites in absorbate	perfusate	S/M[a] for total RA
Fed	58.8 ± 2.7	< 5	1.41 ± 0.05
Starved	74.8 ± 2.2[b]	< 5	1.81 ± 0.08[b]
Starved + acetone	76.1 ± 5.2[b]	< 5	1.73 ± 0.13[b]

[a]Serosal to mucosal concentration ratio of total radioactivity

[b]Significantly different from fed animals, $p < 0.05$

In mice fed ad libitum only 40% of the radioactive material absorbed cochromatographed with authentic NNK indicating a high first pass metabolism of NNK which was further increased by fasting but not by additional treatment with acetone. Three major metabolites could be separated, none of which cochromatographed with 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol, the major metabolite formed in vitro by liver and lung preparations. The evaluation of the toxicological importance of the first pass metabolism of NNK has to wait for the identification of the metabolites formed by jejunal segments.

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11 EFFECTS OF NICOTINE ON INFLAMMATORY MEDIATOR RELEASE AND SIGNAL - TRANSDUCTION

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Various cell types are involved in host defense mechanisms. After activation, the cells such as the granuloctes, monocytes, mast cells, lymphocytes, basophils and platelets are able to generate and release a variety of mediators, e.g. histamine, prostaglandins, leukotrienes and the platelet-activating factor (PAF). The mediator release is thus a part of inflammatory reactions which are either acute or chronic. It is recently known that a variety of environmental agents are able to alter the system of cellular and mediator interaction. It was the purpose of our study to determine the effects of chronic smoke and the in-vitro incubation of granulocytes and platelets with nicotine on lipoxygenase-derived arachidonic acid metabolites and steps of the signaltransduction cascade. To study the effects of cigarette smoke on the amounts of leukotrienes granulocytes from smokers and nonsmokers were incubated with various stimuli- such as Ca-ionophore A23187 and opsonized zymosan. Our data clearly demonstrate that the total amount of LTB4-generation after stimulation of PMNs from nonsmokers with the Ca-ionophore were increased as compared to PMNs of smokers. In contrast, after stimulation with opsonized zymosan, the generated amounts of LTB4 from PMNs of nonsmokers were decreased as compared to PMNs of smokers. Similar results were observed by priming the PMNs of nonsmokers with nicotine-tartrate in vitro. The modulation of leukotriene generation with nicotine-tartarte was clearly dependent on the stimulus: leukotriene B4 generation was inhibited after stimulation with Ca-ionophore, whereas with opsonized zymosan it was enhanced. Only nicotine ditartarte concentrations higher than $1*10^{-8}$M showed these modulatory effects. Incubation of human platelets with nicotine ditartrate (at a wide concentration range under noncytotoxic conditions) did not affect the platelet aggregation, the generation of the chemotactic factor 12-HETE, the metabolism of the platelet activating factor PAF and the release of serotonin. No significant effects on protein kinase C activity (PKC) from smokers and nonsmokers were observed for stimulated and nonstimulated platelets. Our data suggest, that only high concentration of nicotine tartrate modifies the mediator generation in dependency of the subsequent stimulus. Further studies have to elucidate which concentrations of nicotine-tartrate really interact with with the target cells and modulate the cellular responses. For this purpose studies of uptake, analysis of signal transduction as well as cell-cell interaction studies will be carried out.

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12 NICOTINE INDUCED ALTERATION IN PHOSPHATASE ACTIVITY IN PLASMA, LIVER AND KIDNEY OF RATS.

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Acid and alkaline phosphatase activities were estimated in plasma, liver and kidney of male Wistar rats treated with nicotine, to assess the functional significance of toxic reactions of nicotine. Nicotine was administered at a dose of 400 ug kg^{-1} b.wt. s.c. (Rosecrans, J.A. Fred. Proc. 29(2) : 748, 1970) for seven days. The enzyme activities were estimated by the standard method (Bessey et al, J. Biol. Chem. 1946, 164, 321-329), data being statistically analyzed using students "t" test.

Acid phosphatase increased in plasma but decreased in liver and kidney, both values being significant. Alkaline phosphatase significantly increased in plasma and kidney, while in liver the decrease was significant. These findings point to a probable mechanism of action of nicotine, involving changes in enzyme synthesis, overall metabolic status of liver as well as lysosomal damage.

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13 EFFECTS OF SMOKING ON ELASTOLYTIC ACTIVITY, ELASTIN-DERIVED PEPTIDES AND ANTI-ELASTIN-ANTIBODIES IN HUMAN SERUM

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The *aim of the study* was to examine the effects of chronic cigarette smoking on granulocyte elastase activity, elastin-derived peptides and anti-elastin-antibodies in human serum .

200 male students were enrolled in the prospective study; 100 smokers (mean age 26 y.) and 100 nonsmokers (mean age 25 y.). Analysis of the smoking behavior declared in the questionnaire and confirmed by determination of cotinine serum levels revealed three groups: nonsmokers (group I, n= 65), former smokers (group II, n= 40) and active smokers (group III, n = 95).
Anti-elastin-antibodies were analyzed by an Elisa test, elastin-derived peptides with a 'sandwich' version of Elisa test using polyclonal antibodies from goat immunization. Elastase-proteinase-inhibitor-complex was quantified using an Elisa test kit (PMN elastase, Merck Diagnostica Darmstadt). α_2-macroglobulin and α_1-proteinase inhibitor were measured by commercially available kits.

The *results* showed significant differences between smokers and non-smokers concerning the α_1-proteinase inhibitor (group I 1.98 µg/l, group II 2.01 µg/l and group III 2.19 µg/l, $p < 0.001$) and anti-elastin-antibodies (group I 2.4 µg/l, group II 3.4 µg/l and group III 4.4 µg/l, $p < 0.003$). Values of elastin-derived peptides, elastase-proteinase-inhibitor-complex and α_2-macroglobulin did not differ significantly.

In *conclusion*, cigarette smoking is considered to stimulate count and function of granulocytes. Destructive effects of lysosomal granulocyte enzymes are inhibited chiefly by complex formation with α_1-proteinase inhibitor. While direct measurement of granulocyte elastase is difficult, increased levels of elastase-proteinase-inhibitor-complex may be taken as evidence of PMN elastase liberation. In the present study no difference of elastase-proteinase-inhibitor-complex was found between the compared groups. Elastin peptides, formed during the degradation of the elastic fibres are also immediately bound by anti-elastin-antibodies. Thus an increased level of anti-elastin-antibodies may be an indirect indication of accelerated peptid consumption by stimulated elastase activity.
This mechanism may be confirmed by the fact that it is accompanied by a significant increase of the physiological elastase inhibitor α_1-proteinase inhibitor.

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14 OXIDATIVELY MODIFIED LOW DENSITY LIPOPROTEINS INDUCE VASCULAR HYPERRESPONSIVENESS IN HYPERCHOLESTEROLEMIA - MODIFICATION BY CHRONIC NICOTINE APPLICATION?

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Low density lipoproteins (LDL) occur in the arterial wall and may accumulate in oxidized form in atherosclerotic lesions, regions prone to vasospasm. Therefore, we studied the influence of oxidized LDL on vascular tone. Isolated rabbit femoral arteries were perfused with Tyrode's solution. Outer diameter (D) was recorded with a photoelectric device. Human LDL was oxidized by incubation with Cu^{++}. Perfusion of the segments with ox-LDL (100-500 µg/ml) caused no or only weak constrictions (max. 1.5 % of resting D). However, in the presence of noradrenaline, phenylephrine, or serotonin (subthreshold concentrations, no contractile response), ox-LDL caused constrictions (max. 31 % of resting D), correlating with the degree of oxidation. n-LDL (100-1500 µg/ml) had no effect on vascular tone.

In order to develop a model in which influences on vascular ox-LDL content can be studied, rabbits were fed a 0.5 % cholesterol for 16 weeks. Their thoracal aortas were isolated and investigated for lipidperoxid content and responsiveness to various contractile agonists. Preliminary results reveal a close correlation between enhanced lipidperoxid content and enhanced responsiveness to the same contractile agonist as used in the studies with in vitro oxidized LDL.

We then wanted to analyze whether chronic sympathetic activation by nicotine application can increase the diet-induced content of vascular ox-LDL, and consequently the vascular reactivity. Since chronic continuous nicotine infusion induces a progressive desensitization of sympathetic responsiveness to nicotine, we developed a protocol of intermittent, cyclic nicotine application, and a test procedure to assess sympathetic responsiveness to acute nicotine test infusions. Osmotically powered implantable minipumps (ALZET, 2.0 ml vol, 5 µl/hr delivery rate) were filled with vehicle and connected with a small PVC tubing of 1.7 ml total inner volume, filled with alternating volumes of nicotine containing solution (20 µl) and vehicle (40 µl). The sterile tubing was coiled around the pump, and with implantation of the set-up, the osmotic pump pressed the content of the coil in the animals peritoneum, resulting in a 4 hr period of nicotine infusion at a rate of 10 µg/kg·min and an 8 hr nicotine free interval. The lack of nicotine tolerance development by this intermittent protocol was verified in the concious rabbit by monitoring the increase in mean arterial pressure (+ 38 ±6 mm Hg) in response to i.v. nicotine test infusions (10 µg/kg·min, 10 min) under muscarinic blockade by methylatropine (0.5 mg/kg i.v.). By this protocol we shall analyze the influence of chronic nicotine on formation of ox-LDL without interference by tolerance development.

Summary: The enhancement of contractile responses by ox-LDL in in vitro experiments suggest that ox-LDL increase the risk of vasospasm, a conclusion which finds support in our preliminary results made with the hypercholesterolemic animals. A possible correlation with chronic nicotine application will be evaluated by using the here presented method.

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15

Smoking Depresses Adipose Lipoprotein Lipase Response to Oral Glucose

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Adipose tissue lipoprotein lipase was studied in smokers (n = 17) aged 18-47 years and compared to enzyme activity in non-smokers of comparable age (n = 8) and a second time in some subjects 5-9 weeks after cessation of smoking (n = 7). Serum cotinine levels served to validate the smoking status of the subjects. Fasting enzyme activity was similar in smokers and non-smokers, when expressed per 10^6 cells, but was significantly increased when normalized for cell size. When lipoprotein lipase was determined in the same individual 4 hours after an oral glucose load, a significant decrease ($p < 0.002$) occurred in the smokers, while enzyme activity rose in the non-smokers ($p < 0.02$). A tendency for enzyme activity to rise after oral glucose was seen in ex-smokers, which did not reach statistical significance. Even though the mean serum insulin and glucose levels did not differ in the 3 groups of subjects, the % decrease in lipoprotein lipase after oral glucose in smokers was negatively correlated with insulin release into serum in the same subject, i.e., the greater the insulin release, the less the decrease in lipoprotein lipase activity. We would like to propose that the lower body weight in smokers is related to the paradoxical response of adipose tissue lipoprotein lipase to carbohydrate and that the reversal of this behavior contributes to the weight gain often observed after cessation of smoking.

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16 COMPARATIVE EFFECTS OF NICOTINE AND COTININE ON PROSTACYCLIN AND THROMBOXANE SYNTHETASE.

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Cotinine, the major metabolite of nicotine, has been detected in the blood of tobacco smokers in much higher concentrations than nicotine and remained longer after cessation of smoking. Cotinine has been suggested to contribute to the cardiovascular effects of nicotine. Nicotine can modulate prostaglandin synthesis and release. Since thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) are involved in the pathophysiology of cardiovascular system, we studied the comparative effects of nicotine and cotinine on the biosynthesis of PGI_2 and TXA_2. Various concentrations (33 - 260 µM) of arachidonic acid (AA) were incubated with horse platelet microsomes (HPM), source of TXB_2 synthetase or horse aorta microsomes (HAM), source of PGI_2 synthetase. Incubation was carried out at 37°C for 2 min and stopped by addition of 0.2 M citric acid. Compounds under study were incubated with enzyme sources at 37°C for 10 min. TXB_2 and 6-keto $PGF_{1\alpha}$ stable metabolites of TXA_2 and PGI_2 respectively were determined by radioimmunoassay. TXA_2 production was not altered by either nicotine or cotinine. On the other hand, kinetic studies using a Lineweaver-Burk plot of 1/V (reciprocal of 6-keto PG $F_{1\alpha}$ formation rate) versus 1/S (reciprocal of AA concentration), resulted in a Km of 4.7×10^{-4} (M^{-1}) and a Vmax of 9.6×10^{-10} ($min.M^{-1}$). In nicotine pretreated HAM, the slope of regression lines were higher than control values and Vmax increased dose-dependently (Fig 1). The slope of regression lines obtained with cotinine-pretreated HAM however, were lower than the control and Vmax decreased with increasing doses (Fig 2). These data suggest that nicotine inhibits and cotinine stimulates PGI_2 synthesis. The degree of inhibition or stimulation was dependent on the concentration of AA in the medium. The inhibition by nicotine of prostacyclin synthetase is not competitive and does not exclude that nicotine may be also an inhibitor of cyclooxygenase. Furthermore, preincubation of HAM with cotinine (10^{-3} M), abolished the inhibitory effect of nicotine on PGI_2 synthesis. This effect was overcome at high concentration of nicotine (fig 3). Thus it appears that cotinine, the major metabolic byproduct of nicotine, can have beneficiary effects, at least in the cardiovascular system.

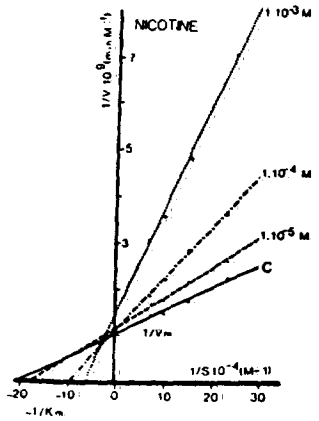

Fig 1

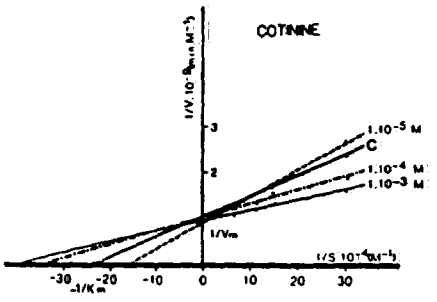

Fig 2

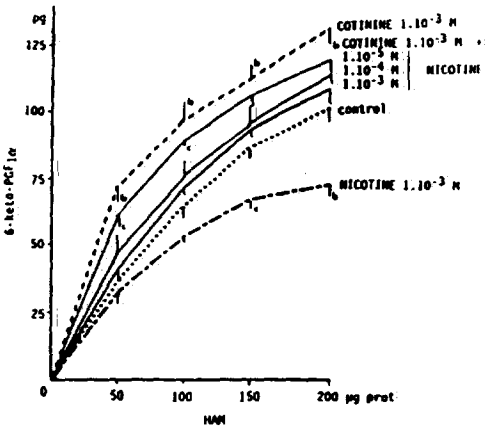

Fig 3

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17

Cardiovascular effects of repeated nicotine administrations: Identification of pharmacologic conditions where self-sensitization or self-blockade takes place

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Tobacco smoking is one of the outstanding risk factors for the production of myocardial infarction and cardiovascular diseases. The tobacco alkaloid nicotine has undesirable effects which predispose to ischemic heart conditions: stimulation of sympathetic ganglia and adrenal glands with increases in heart rate and in blood pressure. There are clinical studies reporting that repeated cigarette smoking produces sustained increases in heart rate (Benowitz et al., 1984). However, classical pharmacology describes the autonomic actions of nicotine as an initial stimulation of autonomic ganglia followed by a more sustained depression. There is, in fact, a general notion in the classical pharmacological literature that the stimulant actions of nicotine show self-blockade (see, for instance, Goldstein et al., 1974).

Our group has previously reported (as have others: Kachur et al., 1986) that in unanesthetized spinal rats the cardiovascular stimulatory actions of nicotine show no self-blockade when nicotine is given in repeated administrations. The purpose of the experiments presented now is to resolve the apparent discrepancy between the concepts of classical pharmacological literature about nicotine self-blockade with the results of clinical studies on repeated cigarette smoking and with our own results of sustained sympathetic stimulation with repeated nicotine administrations.

The effects of repeated injections of nicotine were studied under the following three types of experimental conditions: a) unanesthetized spinal rats; b) spinal rats anesthetized with pentobarbital (60 mg/kg i.p.), and c) intact rats anesthetized with the same dose of pentobarbital.

Nicotine was found to produce dose-related sympathetic effects (25 to 400 µg/kg) in the unanesthetized spinal rat. Pentobarbital markedly reduces sensitivity to the sympathetic stimulant effects of nicotine in the form of a large shift to the right in nicotine's dose-response curves. Self-blockade by nicotine was only observed with extremely high doses 2000 µg/kg and higher) which were only possible to administer in animals under pentobarbital anesthesia; high doses of nicotine produce intracraneal hemorrhages in spinal unanesthetized animals.

In conclusion, in the unanesthetized state, the cardiovascular system is very sensitive to nicotine's sympathetic stimulant effects. The doses of nicotine which produce such stimulant effects are in a range relevant to nicotine concentrations obtained with heavy cigarette smoking. These doses of nicotine do not produce self-blockade but in fact produce self-sensitization. With regard to cardiovascular stimulation, the classical self-blockade by nicotine can be reached only under special conditions of general anesthesia.

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18 Effect of nicotine and cotinine on arterial blood pressure of spontaneously hypertensive rats

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It has been known for a long time that smoking tobacco induces peripheral vasoconstriction. Similarly, the intravenous administration of nicotine stimulates the sympathetic ganglia and the adrenal medulla leading to sympathomimetic effects and resulting in vasoconstriction, tachycardia and elevated blood pressure. An involvement of the arterial chemoreceptors has also been described. However, several reports on a blood pressure reducing effect of high nicotine doses (>3 mg/kg.day) or after prolonged administration of the alkaloid (several weeks) have been published recently. The experiment reported here was performed to establish whether a high nicotine dose reduces the high blood pressure of spontaneously hypertensive rats (SHR), and whether this effect is due to the metabolite cotinine rather than to nicotine. Three groups of SHR rats were used. The control group (C, n=5) received tap water as drinking fluid. The rats of the other groups received nicotine (N, N=6, 4 mg/kg BW.day) or cotinine (Cot, n=6, 1 mg/kg BW.day) for two weeks. Arterial blood pressure was measured by tail plethismography before and twice weekly after starting drug administration.

The N rats gained only 8 g body weight (284±8 g to 292±7, x±SD), whereas the C rats gained 15 g (281±11 to 302±15) and the Cot rats 16 g (281±13 to 297±12). Blood pressure before treatment was: C=221±14 mmHG, N=219±7, Cot=200±17. At the end of the first week of treatment the blood pressure was 228±6, 216±7 and 206±16 mmHG, and at the end of the second week 202±15, 197±8 and 197±8 respectively. Two additional rats were given 10 mg/kg BW.day cotinine. These animals had a mean body weight gain of 15 g (269, 285 to 283, 300 g). Their blood pressure was 231 and 232 mmHG before and 190 and 204 after two weeks of treatment.

It is concluded that oral administration of nicotine or cotinine does not significantly affect the blood pressure of spontaneously hypertensive rats.

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19 DIFFERENTIAL EFFECTS OF NICOTINE ON CARDIAC NORADRENALINE AND NEUROPEPTIDE Y RELEASE IN NORMOXIA AND ANOXIA

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Harmful cardiovascular actions of cigarette smoking have been attributed to a sympathetic activation of the heart. Hence, the effect of nicotine on cardiac release of noradrenaline, dihydroxyphenylethyleneglycol (DOPEG) and neuropeptide Y was investigated in isolated perfused guinea pig hearts. Neuropeptide Y is a sympathetic peptide transmitter, which is co-stored with noradrenaline. Common release of noradrenaline and neuropeptide Y is known to specify an exocytotic release mechanism (Arch Pharmacol 340: 509, 1989). Overflow of DOPEG, the major intraneuronal metabolite of noradrenaline, indicates a rise of free intracellular noradrenaline concentrations, which characterizes nonexocytotic noradrenaline release (Circ Res 60: 194, 1987). The latter release mechanism is not accompanied by neuropeptide Y overflow. Noradrenaline and DOPEG were determined by HPLC, neuropeptide Y was measured by radioimmunoassay.

In normoxia, nicotine (10-1000μM) induced a concentration dependent release of both noradrenaline and neuropeptide Y (IC50: 30μM). The release of noradrenaline and neuropeptide Y was positively correlated (r=0.81) with a molar ratio of about 1000/1. The release of both transmitters was dependent on extracellular calcium and underwent rapid tachyphylaxis. No DOPEG overflow was detectable during nicotine administration in normoxia.

During energy depletion by anoxia or cyanide intoxication and glucose-free perfusion, spontaneous noradrenaline overflow occured after 15 min and reached peak levels after 40 min (160 pmol/g/min). In presence of low nicotine concentrations (3μM) anoxic noradrenaline release started after 10 min with rapid increase at 20 min (250 pmol/g/min). In these experiments noradrenaline release was independent of extracellular calcium and did not coincide with neuropeptide Y release. Nicotine was present from the beginning to the end of anoxia without arising tachyphylaxis. In cyanide intoxication noradrenaline release was preceded and accompanied by DOPEG overflow. Nicotine (3μM) caused an acceleration of both noradrenaline and DOPEG overflow.

Summary: In normoxia, nicotine induces exocytotic release of noradrenaline and neuropeptide Y. In anoxia, nicotine accelerates anoxic noradrenaline release at concentrations, which are ineffective in normoxia, by a nonexocytotic mechanism.

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20 Evidence for noradrenaline-mediated changes in platelet and heart function following chronic treatment of rats and guinea pigs with nicotine

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Introduction: Platelets of rats chronically pretreated for 8 weeks with nicotine (10mg/kg/day, applied from subcutaneously implanted osmotic minipumps) exhibited reduced sensitivity towards the aggregatory stimulant ADP [Becker et al. (1988) Klin Wochenschr 66 (Suppl XI),28 - 36]. This action of nicotine could be prevented by simultaneously applying the ß-blocker propranolol, was not mimicked by adrenaline, and was not elicited in guinea pigs, a species known to lack thrombocytic adrenoceptors. A species specific effect of nicotine was also found when hearts isolated from pretreated animals performed pressure-volume work under standardized conditions [Becker and Gerlach (1988) in: Rand and Thurau (eds) The pharmacology of nicotine. IRL Press, Oxford, pp 140]. Hearts of guinea pigs exhibited a transitory reduction in performance after 1 - 2 weeks of nicotine application, whilst longer periods of pretreatment (8 - 12 weeks) improved heart function vs controls. No such effects were noted for the rat.

Problem and Approach: To establish whether the described actions of nicotine were due to a selective, chronic elevation of the noradrenergic tone, platelet function was assessed in rats chronically treated with noradrenaline (0.05 and 0.5 mg/kg/day for 8 weeks). In other sets of experiments, the density of ß-adrenoceptors was determined in hearts of guinea pigs and rats after various periods of nicotine application. Moreover, the myocardial tissue levels of high energy phosphates (ATP, CrP) were measured following isolation of pretreated guinea pig hearts to ascertain the degree of metabolic stress.

Results: i) Chronic application of noradrenaline to rats blunted platelet responsiveness towards ADP to the same extent as did nicotine. ii) The ß-adrenoceptor density of rat hearts declined rapidly (-50% after 1 week of nicotine), but then remained constant. Since only the density of control hearts showed a very strong, growth-dependent drop, densities of controls and nicotine treated hearts were about the same again after 8 weeks, albeit at a lower level. In contrast, ß-adrenoceptor density in hearts of guinea pigs fell more slowly during nicotine application; there was no reduction relative to controls after 1 week, however a 40 to 50% decline had developed after 4 and 8 weeks. iii) In hearts treated for 2 weeks with nicotine, ATP levels and work performance proved to be lower than in controls by about 10% and 20%, respectively, but there was no difference after 6 weeks.

Conclusions: Chronic application of nicotine to rats and guinea pigs seems to generally enhance ß-adrenoceptor mediated effects by elevating noradrenaline levels. In rats this leads to a reduction in blood platelet activity. However, the rat heart is protected from any untoward metabolic stress due to rapid down-regulation of ß-adrenoceptor density. Since the down-regulation proceeds more slowly in the guinea pig, it may be assumed that full sympathetic stimulation of the heart is still possible after about 2 weeks of treatment, cardioprotection only becoming manifest after about 4 weeks of nicotine application. **In summary,** effects of nicotine clearly vary with the animal species, the organ or cell type, and the duration of chronic application.

Supported by a grant from the German Research Council on Smoking and Health

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Source: https://www.industrydocuments.ucsf.edu/docs/pspk0000

21 EFFECT OF NICOTINE ON RED CELL DEFORMIBILITY IN DIABETES MELLITUS

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The property of deformibilty of the red cells is very important in the maintenance of microcirculation. It was well established that in diabetes mellitus the red cell deformibilty was reduced. In this study we investigated the effect of nicotine (10^{-5}M-10^{-2}M) on erythrocyte deformibilty in non-insulin-dependent diabetes mellitus.

Blood samples obtained from 15 healthy volunteers(fasting blood glucose: 85±7) were used as control and 28 diabetic patients(fasting blood glucose:265±46, with mean±SD duration of diabetes 6±2 years) were used as experimental groups. Erythrocyte deformibity was assessed in terms of erythrocyte filtration time and erythrocyte filtration time was measured by microfiltration technique.

The mean±SD duration of filtration time in control group was 6.2±1.2 sec in diabetic group was 8.4±1.5 sec.

Nicotine was added to the solutions of 20% red cell suspension to yield final concentrations of 10^{-5}M,10^{-4}M,10^{-3}M and 10^{-2}M and the measurements of filtration times were made 0.5 min.,1 min.,2 min.,3 min.,5 min. and 10 min. after addition of nicotine.

Our results showed that the mode of action of nicotine both on control and diabetic groups is time-dependent and the intensity of its action is concentration -dependent. Initially it caused a decrease in filtration rate which lasted 2-3 min at lower concentrations(10^{-5}-10^{-4}M),3-4 min at higher concentrations(10^{-3}-10^{-2}M). At lower concentrations the decrease in filtration rate was 16% and 22% in control and diabetic groups respectively.At higher concentrations these values turned out to be 21% and 29% in control and diabetic groups. The initial fall in deformibility was transient and it was followed by significant enhancements. At the 5^{th}min. the durations of filtration were 10% and 18% shorter than their pre-nicotine values in control and diabetic groups respectively. At the 10^{th}min measurements more or less the same values as at the 5th min were obtained.

Conclusion: Nicotine alters the deformibilities of both healthy and diabetic red blood cells in a time- and concentration-dependent manner,but the effect on diabetic cells is more pronounced.

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22 COMPARISON OF METHODS FOR REMOVAL OF EPITHELIUM IN AIRWAYS FROM A SMALL ANIMAL. NEED FOR HISTOLOGIC VERIFICATION.

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A study on the effect of the epithelium on nicotine-induced bronchoconstriction (Lang, Hahn 1990) directed our attention to the need for a defined and histologically controlled method to remove the epithelium. Systematic histologic examination revealed the ability of some methods to cause substantial damage to the basement membrane and into subepithelial tissue. We therefore initiated a formal study of different methods for removal of epithelium in the ferret trachea.

We examined 4 techniques of epithelium removal in tracheae excised from ferrets under pentobarbital anaesthesia: (1) gentle scraping with a scapel blade moved backwards (away from the cutting edge), (2) moving a Q-tip through the unopened tracheal tube without lateral pressure, (3, 4) stroking the mucosa of opened tracheal segments with a Q-tip, exerting (3) light or (4) moderate pressure.

Method (1) removed the epithelium and most of the basement membrane, caused damage to the submucosa in most specimens and was abandoned without quantification. Each of the remaining methods was quantified in 6-8 segments by each of two observers. Since no consistent differences were noted between observers results from different observers were pooled. The table lists structures left intact by procedures (2), (3) and (4).

Method	Epithelium %, x±SE	Basement Membrane %, x±SE	Basal Cells mm^{-1}, x±SE
(2)	0.0 ± 0.0	71 ± 10	17 ± 3.0
(3)	3.1 ± 2.2	63 ± 06	13 ± 2.7
(4)	1.6 ± 0.7	41 ± 06	12 ± 3.5

Method (2) was as complete as methods (3) and (4) in removing the epithelium (efficiency of removal for all methods, 97-100%), but caused less damage to the basement membrane than the other two methods, especially method (4) which removed almost 60% of the basement membrane compared to 29% removed by method 2. In the ferret, method (2) was clearly best, followed closely by method (3). Preliminary studies on tracheae of large animals have shown a different pattern of results in that the mucosa from large animals was more resistant to removal of the epithelium. However, damage to the basement membrane was also very frequent. Complete removal of epithelium without damage to the basement membrane and/or to subepithelial connective tissue was as difficult to achieve in large as it was in small animals. We conclude that each method employed for removal of epithelium needs to be quantified histologically. Functional studies will be most difficult to interpret without such control.

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23 NICOTINE-INDUCED SUBMUCOSAL GLAND SECRETION AND AIRWAY SMOOTH MUSCLE CONTRACTION HAVE DIFFERENT MECHANISMS.

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We have demonstrated strong effects of nicotine on airway gland secretion and on airway smooth muscle contraction. These effects may be due to stimulation of nicotinic receptors in ganglia and along postganglionic nerves or to a direct effect of nicotine on receptors in the end organ. We studied these two possible mechanisms in 2 series of experiments, one on glands and one on airway smooth muscle.

Studies on glands: We mounted tracheal segments from 76 ferrets (37 M, 39 F), mean weight 915±314 g in Ussing chambers. We applied the radioactive precursor $Na_2{}^{35}SO_4$ to the submucosal side of tracheal segments and measured ^{35}S in secretions obtained from the luminal side. We studied nicotine-induced mucus secretion in the presence and absence of the neural blocker tetrodotoxin (TTX) using a concentration (10^{-6}M) that blocked the secretory effect of electric field stimulation completely. We also examined the inhibitory effect of atropine (10^{-7}-10^{-4}M) and of hexamethonium (10^{-9}-10^{-4}M) on nicotine-induced secretion.

Studies on airway smooth muscle: We performed similar studies on tracheal rings from 18 ferrets (10 M, 8 F), mean weight, 1216±511 g. Muscle studies were performed in the same medium and under the same conditions as secretion studies. Antagonists tested against nicotine-induced muscle contraction were tetrodotoxin (10^{-6}), hexamethonium (10^{-6} M, 10^{-5} M) and atropine (10^{-8} M, 10^{-7} M, 10^{-6} M).

While tetrodotoxin inhibited strongly or blocked, nicotine-induced muscle contraction tetrodotoxin did not reduce nicotine-induced mucus secretion significantly (477±61 vs. 342±74%, $p<0.2$, without vs. with TTX, n=15). While the dose of atropine that blocked nicotine-induced muscle contraction was exactly the same as the dose that blocked ACh-induced muscle contraction doses of atropine blocking nicotine-induced mucus secretion were three orders of magnitude (1000x) higher than those required to block ACh-induced mucus secretion. Thus, at these doses atropine acted nonspecifically as a nicotinic (as well as muscarinic) blocking agent. Only results with hexamethonium were comparable between the 2 studies in that the same concentration of hexamethonium inhibited (10^{-6} M) or blocked (10^{-5} M) nicotine-induced muscle contraction and nicotine-induced gland secretion. Thus both effects of nicotine, nicotine-induced muscle contraction and nicotine-induced gland secretion, were mediated by nicotinic receptors because they were blocked by the specific nicotinic receptor antagonist hexamethonium. However, only nicotine-induced muscle contraction was mediated by neural pathways and was therefore TTX sensitive and was blocked by concentrations of atropine causing specific muscarinic blockade. In contrast, nicotine-induced gland secretion was caused by a direct effect of nicotine on nicotinic receptors in the end organ. It was therefore not TTX sensitive and was not blocked by concentrations of atropine causing specific muscarinic ACh receptor blockade.

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24 NICOTINE-INDUCED CONTRACTION OF TRACHEAL SMOOTH MUSCLE *IN VITRO* REQUIRES PRESENCE OF TRACHEAL EPITHELIUM.

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The tracheobronchial epithelium can inhibit airway smooth muscle contraction by various mechanisms but epithelial cells in culture have also been shown to release mediators capable of causing bronchoconstriction. In order to assess the mechanism of and the rôle of the epithelium in, nicotine-induced bronchoconstriction we suspended tracheal smooth muscle strips or tracheal rings from 22 ferrets (1247±526 g, x±SD) anaesthetized with 66±19 $mg \cdot kg^{-1}$ pentobarbital in organ baths filled with medium M 199 and aerated with 95% O_2 and 5% CO_2. Muscle tension was measured with force transducers (FT03, Grass). Signals were amplified and recorded with an eight channel recorder (Hellige). Baseline smooth muscle tension was set at a level leading to maximal contraction on subsequent addition of acetylcholine (ACh, 10^{-6}M). To determine magnitude and mechanism of nicotine-induced bronchoconstriction we constructed a cumulative dose response curve to ACh (10^{-8} - 10^{-4} M) after which each ring received a single dose of nicotine ($3 \cdot 10^{-5}$ - $3 \cdot 10^{-4}$ M) in the presence or absence of hexamethonium (10^{-5} M, 10^{-6} M), tetrodotoxin (10^{-6} M) or atropine (10^{-8}, 10^{-7}, or 10^{-6} M). To determine the rôle of the epithelium in nicotine-induced bronchoconstriction we removed the epithelium from alternate tracheal strips by stroking them gently with a scalpel blade moved backwards (i.e., away from the cutting edge). This procedure also removed some subepithelial connective tissue (verified histologically). Dose response curves to ACh and nicotine were established for pairs of tissues with and without epithelium, each pair receiving only one dose of nicotine.

Nicotine induced brief muscle contractions not exceeding 25% of the ACh-induced maximum. Contractions were blocked by hexamethonium, atropine (10^{-7} M, 10^{-6} M), and were abolished or inhibited strongly by tetrodotoxin, suggesting the involvement of nicotinic ganglionic and/or neuronal receptors and of muscarinic smooth muscle receptors. Removal of epithelium strongly inhibited contractions at concentrations of nicotine $> 3 \cdot 10^{-5}$ M which completely removed any dose response effect. ACh-induced contractions were not changed by removal of epithelium, demonstrating smooth muscle integrity.

We conclude that nicotine acts on ganglionic and/or neuronal nicotinic receptors to initiate muscle contraction by a neural pathway. The effect can be blocked either by the neuronal blocker tetrodotoxin or by atropine which antagonizes the contracting effect on smooth muscle of Ach released from the nerve ending. Removal of epithelium and/or of portions of the submucosal tissue inhibits or blocks nicotine-induced smooth muscle contraction presumably by removing or interrupting nerves running in or close to the epithelium. This reveals one more mechanism by which the removal of epithelium can influence smooth muscle contraction. The epithelium and the adjacent subepithelial tissue influences airway smooth muscle contraction not only by generating relaxing or contracting factors or by acting as a diffusion barrier but also by acting as a nerve conduit. These could be either efferent cholinergic nerves or afferent (e.g., peptide containing) nerves interacting locally with the efferent pathway.

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25 Effect of chronic nicotine consumption on the morphology of the rat parotid gland.

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The effect of chronic nicotine consumption on the morphology of the parotid gland was studied in male Wistar rats. Nicotine was administered orally via the drinking water (100mg /l).

Within an exposition period of 90 days nicotine caused distinct morphological alterations in comparison to pair-fed control rats. Light microscopic and electron microscopic investigations as well as a morphometrical analysis showed

- a swelling of the acinar cells
- an increased number of enlarged, light, immature secretory granules within the acinar cells.
- an increase of the granular endoplasmatic reticulum
- an enlargement of the Golgi complexes

and an edematous swelling of autonomous intraglandular nerves.

These morphological findings especially the increase of the granular endoplasmatic reticulum, the enlarged Golgi complexes and the large secretory granules reflect a chronic stimulation of protein synthesis and protein secretion . Similar morphological alterations have been observed in the acinar cells of parotid glands of rats being chronically treated with the ß-adrenergic drug isoproterenol and been paraphrased by the medical term sialoadenosis.

It seems to be likely that the observed morphological alterations of the rat parotid gland after nicotine loading are due to a chronic stimulation of glandular ß-adrenoceptors via a nicotine induced release of catecholamines from the adrenals.

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26 Effects of nicotine on ureteric motility

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The effects of nicotine on the ureteric motility of the sheep were studied in vitro. The ureters were obtained early in the morning from the local abattoir. Ureteral strips were suspended vertically in 10 ml organ baths filled with De-Jalon solution maintained at 37° C and gassed with 95% oxygen and 5% carbon dioxide. The ureter has adrenergic and cholinergic innervations. Sensory nerves, however, may have important functions in ureteric motility regulation. It's motility is affected by these innervations and prostaglandins. Indomethacin (10^{-5} M) inhibited and finally stopped rhythmich motility. ACh (10^{-8} - 10^{-4} M) increased dose-dependantly both frequency and amplitude of contractions. Nicotine (10^{-6} -10^{-3} M) caused stimulatory effect on frequency and amplitude of contractions.

Nicotine may act on the nicotinic receptors or/and produces stimulating effects in the adrenergic nerve terminals on ureters.

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27 Craniofacial and Cardioteratogenic Effects of Nicotine in Mouse Embryos

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During the past few decades, it has become increasingly evident that human embryos are subject to a variety of enviromental influences that might have deleterious effects on their development. these enviromental agents can alter the normal course of embryogenesis. In this investigation, effects of nicotine on heart and craniofacial development in mouse embryos from early days of gestation through the first few post natal days was studied by means of histochemical and scanning electron microscopy methods. We were able to produce a spectrum of craniofacial defects (cleft lip with or without related cleft of palate , CL/P) and cardiac malformations(endocardial cushion type of defects) by exposing the mother to nicotine or smoke from high nicotine cigarettes, NIH code 32,. The critical time of development was determined and nicotine was injected intraperitoneally at different concentrations. The dosage of 1 mg/kg of nicotine on the morning of gestational day 10 (11:00 a.m.) was the most effective and was used for the greater part of this research. All doses were administered in a volume of 25 Ul. Controls were injected with an equal volume of diluent (sterile o.9% NaCl). Day 10 of gestetion (vaginal plug =day 0) is prior to the time that mesenchymal cells migrate into the cardiac jelly (CJ) of the endocardial cushions just before the cardiac septa develop.

Intrapritoneal injection of 1 mg/kg of nicotine, produced 32% (47/148) of embryos or newborns with cardiac defects and 20% (30/148) of these also showed CL/P. When mesenchymal cells of the CJ and the facial processes were examined two days after nicotine exposure, striking changes in cell shape and size were evident.In-as-much as the incidence of mesenchymal changes in the CJ and in the mesenchyme of the facial placodes seen at day 12 (two days after exposure of nicotine) parallels the incidence of malformations seen in newborns. It appears that the change morphology of these cells may reflects a failure of normal tissue interactions. It is possible that nicotine affects the glycosaminoglycans (GAG)a prominent class of extra cellular matrix macromolecules which are important in many developmental phenomena such as cellular interactions , cell shape , differentiation, etc. We also conclude that critical stages of development occur in the heart,facial processes at the same time and therefore certain teratogens such as nicotine may interfere with normal development of several organ systems concurrently.

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28 PRENATAL INTERACTION OF NICOTINE WITH DEVELOPING NERVOUS AND ENDOCRINE SYSTEMS

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Nicotine may affect the developing brain either through direct actions on developing nervous tissue or via effects on endocrine systems and metabolism. We focussed on developing central transmitter systems and neuroendocrine regulation of the Long Evans rat. Specific interactions of nicotine with developing neurons can be expected when nicotinic cholinergic receptor sites are formed. In vitro autoradiography reveals binding sites for (3H)nicotine in lower brainstem and spinal cord at gestational day (GD) 12, i.e., at the end of the embryonic period (Lichtensteiger et al., Progr. Brain Res. 73, 137, 1988). Later, they appear in anterior brain regions including cerebral cortex (GD 18). In cortex, a shift of maximum density from deep layers (VI) to layers IV+III occurs during the second and third postnatal week. As nicotinic receptor distribution changes throughout ontogeny and differs markedly from the adult condition, the effects of nicotine on a particular brain region may be quite different depending upon the developmental stage.

Effects of nicotine on nicotinic binding sites were studied in fetuses and offspring of time-pregnant rats implanted at GD 12 with osmotic minipumps delivering 0.25 mg/kg x hr during one week. At GD 21, quantitative autoradiography reveals a marked increase in density of (3H)nicotine binding sites in all brain areas studied, including substantia nigra, striatum and deep cortical layers. After delivery, receptor density returns to normal levels in most areas. However, in young adults (2 1/2 months), receptor density remains slightly elevated in cerebral cortex and has decreased to below control level in substantia nigra. The pattern in cortex and nigrostriatal system changes again until old age (20 months) which suggests differences in ageing processes.

Nicotinic receptors develop in close relation to pontine and mesencephalic catecholamine neuron groups of the fetus. Catecholamine metabolism of fetal forebrain is differently affected by acute and chronic nicotine administration (Ribary and Lichtensteiger, J. Pharmacol. Exp. Ther. 248, 786, 1989). Chronic prenatal exposure initiates a complex pattern of alterations in central dopamine and norepinephrine metabolism which changes from fetal through postnatal to adult life and differs between sexes. The pattern seen at PN 15 can tentatively be related to activity changes of male offspring (Schlumpf et al., Pharmacol. Biochem. Behav. 30, 199, 1988) in terms of the dopamine hypothesis of MBD.

Nicotine interferes with the organization of fetal neuroendocrine axes. In the male GD 18 rat fetus, it suppresses a characteristic rise in plasma testosterone and, moreover, increases corticosterone. This is followed by changes in the activity of brain steroid aromatase which converts androgens to estrogens and is important for sexual brain differentiation. Enzyme activity (measured by conversion of (3H)androstenedione) is reduced to female levels in male offspring at PN 5. This could impair organizing actions of androgens on the developing brain. In adult offspring, sexual dimorphism of saccharin preference is lost and male sexual behavior affected.

Thus, prenatal nicotine exposure initiates a complex interplay between effects on developing nervous and endocrine systems which continues after cessation of drug treatment.

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29 DESIPRAMINE INHIBITS THE NICOTINE-INDUCED EXOCYTOSIS IN RAT PHAEOCHROMOCYTOMA CELLS (PC12)

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Already in 1970, Su and Bevan (J Pharmacol Exp Ther 175: 533-540) reported the tricyclic antidepressant desipramine to inhibit the nicotine-induced release of noradrenaline from adrenergic neurons. However, the mechanism of inhibition has not yet been clarified. We investigated the effect of desipramine on the exocytotic release of ^{3}H-noradrenaline in a clonal cell line (PC12) which expresses many properties characteristic of adrenergic neurones.

The PC12 cells were grown in surface culture on plastic dishes coated with collagen. Prior to the experiment, the cells were differentiated by nerve growth factor (200 µg/l for 3 days). The cells were loaded with ^{3}H-noradrenaline (10 nmol/l) for 60 min. After a wash-out period of 30 min the release of ^{3}H-noradrenaline into the incubation medium was stimulated by various methods. The fraction of ^{3}H-noradrenaline (determined after column chromatography) which appeared in the incubation medium during 1 min of stimulation was measured. (1) Depolarization of the cells by potassium (56 mmol/l) released 3.3 ± 0.2 % (n=11) of the intracellular ^{3}H-noradrenaline, (2) the calcium-ionophore A23187 (10 µmol/l) released 1.8 ± 0.2 % (n=7) and (3) nicotine (100 µmol) released 6.0 ± 0.4 % (n=29). Irrespective of the method by which release of ^{3}H-noradrenaline was induced, the release of ^{3}H-noradrenaline absolutely depended on the presence of extracellular calcium. The nicotine-induced release was markedly inhibited by 30 µmol/l hexamethonium but not by 0.5 µmol/l atropine. In some experiments, the release of neuropeptide Y (NPY) - an indicator of exocytosis - was measured by radioimmunoassay. The fraction of NPY which has been released by depolarization (56 mmol/l potassium) and by nicotine (100 µmol/l) corresponded to the release of ^{3}H-noradrenaline. Thus, clonal PC12 cells are a useful model to study exocytosis elicted by the stimulation of nicotinic acetylcholine receptors.

Desipramine potently inhibited the nicotine-induced release of ^{3}H-noradrenaline, the IC_{50} being as low as 61 nmol/l (95% confidence interval: 35, 107; n=6). At 1 µmol/l, desipramine inhibited the nicotine-induced release of NPY and the nicotine-induced rise of intracellular calcium by 97 ± 2 % (n=3) and 89 ± 4 % (n=8), respectively. Intracellular calcium was measured fluorimetrically by the fura-2 method. On the other hand, desipramine was a far less potent inhibitor when the release of ^{3}H-noradrenaline was elicted more directely by depolarization (56 mmol/l potassium) or by a calcium-ionophore (10 µmol/l A23718), both IC_{50}'s being above 3 µmol/l. The results suggest, that desipramine inhibits nicotine-induced exocytosis at an early step in the sequence of events which eventually leads to exocytosis. Most probably, desipramine blocks the nicotinic acetylcholine receptor or the cation channel which is closely associated with that type of receptor.

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30 Nicotine Infusion in Man Stimulates Plasma-Vasopressin, -ACTH and -Cortisol in a Dose Dependant Manner

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Nicotine has an antidiuretic effect, which is supposed to be mediated by vasopressin (Husain et al). According to Goldsmith et al. vasopressin release is stimulated by nicotine only if nausea occurs. Rowe et al. infused nicotine into healthy subjects in order to obtain nicotine plasma levels similar to those during smoking. While smoking stimulated vasopressin release, nicotine infusion failed to do so. The authors concluded that vasopressin stimulation due to smoking was mediated by an airway-specific mechanism.

The present study investigates the response of plasma-vasopressin (AVP), -ACTH and -cortisol to nicotine infusion in man. Seven male, healthy, non-smoking subjects were each studied three times, receiving nicotine infusions of 0.5 , 1.0 and 2.0 µg/kg body weight/min respectively over 20 minutes on different days. Blood pressure and heart rate were measured frequently before and during infusion. All symptoms reported by the subjects were carefully protocolled. Blood was drawn twice at baseline and 5, 10, 15, 20, 25, 30, 40, 55 and 70 minutes after starting the infusion. Plasma AVP, -ACTH and -cortisol were measured using specific radioimmunoassays, plasma nicotine by gas-chromatography after extraction.

Plasma nicotine rose during infusion in a dose-dependant manner to peak levels of about 5, 10 and 20 ng/ml respectively in the three experiments. The lowest dose of nicotine caused mild lightheadedness and a feeling of arousal in three subjects but no changes in hormone levels. Infusion of the medium dose led to lightheadedness in most subjects and to mild nausea in one. There was a clear-cut rise of the three hormones measured. 2.0 µg/kg b.w./min of nicotine led to nausea in four subjects and to lightheadedness in the others. AVP, ACTH and cortisol rose more pronounced than in the medium dose group.

Thus, intravenous nicotine infusions led to dose-dependant increments of plasma nicotine, -AVP, -ACTH and -cortisol. The threshhold for the stimulation of all parameters measured is between 0.5 and 1.0 µg/kg b.w./min of nicotine infusion. The occurance of nausea was not a prerequesite for the stimulation of AVP release. The activation of all variables measured in this study at a similar threshold dosage of nicotine suggests a common basic mechanism at a nicotinergic receptor site. It is also possible that stimulation of AVP and ACTH by the same threshold dose of nicotine reflects corticotropin (ACTH) release by AVP.

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31

A single dose of nicotine may be sufficient to induce tyrosine hydroxylase in noradrenergic neurones.

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We have recently reported that nicotine given to rats increases catecholamine synthesis, in a regionally selective manner, in brain areas in which increased catecholamine release can be demonstrated with *in vivo* techniques (1,2). We have also shown (3) that repeated daily injections of nicotine for between 3 and 28 days increase the activity of tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, in noradrenergic neurones. The early response seen in the cell bodies in the locus coeruleus is followed by increases in the terminal regions (cerebellum, hypothalamus, hippocampus and frontal cortex) over a time course related to the distance of the terminals from their cell bodies. The increase in TH activity in the terminals seems to be independent of continued nicotine administration once an increase in enzyme activity in the locus coeruleus is established with 7 days of treatment (4). We now report the effect of a single dose of nicotine on TH activity in these neurones over a similar time scale.

Nicotine bitartrate (0.8mg/kg as free base), neutralised in isotonic saline, or saline alone was given to groups (n=6) of male Sprague-Dawley rats by subcutaneous injection. Animals were sacrificed on the seventh day after injection for the cell body regions or on the 21st day for the terminals. The locus coeruleus, hypothalamus and hippocampus were removed by dissection from slices prepared using razor blades and an aluminium guide block, then frozen in liquid nitrogen pending analysis. Samples were thawed and homogenised, then assayed for TH activity using a method described in (2) in which ^{3}H-tyrosine was supplied as substrate, 6-methyltetrahydropterin as cofactor and ascorbate as reductant. The product, ^{3}H-DOPA, was isolated on alumina columns.

A single injection of nicotine on the first day or on the first two days resulted in TH activities in the locus coeruleus 7 days later of 232% and 250% of saline controls respectively. This compares with an increase to 223% of controls after 7 days of repeated nicotine administration (3). 21 days after a single dose of nicotine, TH activities had increased to 157% of controls in the hippocampus and to 136% in the hypothalamus, compared with 180% and 147% respectively following 21 days of repeated nicotine injection.

The effects of chronic nicotine on TH activity in noradrenergic neurones are analogous to those after a single dose of reserpine (5). Reserpine has been shown to increase TH mRNA (6), suggesting that induction in the cell bodies followed by axonal transport, rather than activation, underlies the increase in enzyme activity. These results imply that subchronic administration of nicotine, or as few as one or two doses, are as effective as chronic nicotine in increasing TH activity. We have observed (3) that the increases in enzyme activity are not sustained even with chronic nicotine administration. This might be due to desensitisation after initial exposure to the drug.

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32 THE EFFECT OF ACUTE AND REPEATED NICOTINE INJECTIONS ON BRAIN DOPAMINE ACTIVATION: COMPARISONS WITH MORPHINE AND AMPHETAMINE.

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Acute systemic injections of morphine, amphetamine and nicotine elicit increased locomotion in rats and repeating these injections produces an enhanced or sensitized locomotor effect. Different lines of evidence suggest that mesolimbic dopamine (DA) activation underlies the acute locomotor effects of these drugs and that enhanced activity in this system is responsible for sensitization to the locomotor effects of morphine and amphetamine. Relatively little is known, however, about the effects on DA activation of repeated injections of nicotine.

We have found that acute injections of (-)-nicotine bitartrate (0.4-0.8 mg/kg, base, s.c.) produce substantial increases (41-49%) in DOPAC/DA in the nucleus accumbens (N.Acc.) and more moderate increases (26-37%) in the antero-medial striatum. Surprisingly, repeating these injections reduced (to 15-26% in N.Acc.) or abolished (in antero-medial striatum) these increases in DA turnover. Interestingly, the opposite effect was obtained in the medial prefrontal cortex (mPFC). Small increases in DOPAC/DA produced by acute injections (18%) gave way to larger increases (31%) after repeated injections. In comparison, acute morphine produced large increases in DOPAC/DA in the N.Acc. (59%) and the mPFC (61%). Unlike with nicotine, however, repeating these injections substantially enhanced the effect of morphine in the N.Acc. (96%) but slightly reduced it in the mPFC (52%).

These findings suggest that nicotine differs from morphine (and amphetamine) in the manner in which it produces enhanced locomotor effects following repeated injection and possibly in its relation to DA as a mediator of reward. Indeed, repeated nicotine reduces the acute activating effects of this drug in subcortical DA terminal fields and increases it in the mPFC while repeated morphine increases these effects in subcortical sites and produces small decreases or no changes in the mPFC.

Results from further experiments suggest that the differential effects of these drugs on DA activation may also have consequences on the regulation of mu-opiate receptors in subcortical DA terminal fields. Preliminary results obtained by quantitative autoradiography indicate that mu-opiate binding sites in these areas are upregulated following repeated injections of amphetamine but not of nicotine. These effects may be related to the sensitization of subcortical DA function induced by repeated amphetamine (and morphine) but not by nicotine.

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33 Effects of pre- and/postnatal treatment with nicotine or postnatal exposure to cigarette smoke on hypothalamic catecholamine nerve terminal systems and neuroendocrine function in adult male and female rats.

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1. Male or female Sprague-Dawley rats were treated with nicotine during the prenatal and the first three postnatal weeks (the pregnant and lactating rats were given nicotine (+) tartrate (165mg/kg) in the tap water). Following one week of withdrawal (age 4 weeks) from pre- and postnatal treatment with nicotine an increased catecholamine (CA) utilization was observed in the medial (MPZ) and lateral (LPZ) palisade zones of the median eminence (ME) of both the male and female rats. This change maintained also at an age of 6-7 months, most prominent in the female diestrous rat and to a lesser degree in the male rat. In the adult male rat also increased noradrenaline (NA) stores were found in the paraventricular hypothalamic nucleus (PA). In the 4 week female rat reduced serum prolactin levels were found following pre- and postnatal nicotine treatment, an effect which was normalized in the adult rat. In the male rat no changes in serum TSH, prolactin, LH and corticosterone levels could be observed at an age of 4 weeks, but increased LH serum levels were found in the 6 month old rat.
2. Male rats were exposed to the smoke from 2 cigarettes (Kentucky reference IR-1 type) every morning from the day 1 after birth for a period of 5, 10 or 20 days. The rats were decapitated 24 h, 1 week or 7 months after last exposure. Serum LH levels were increased 24 h after a 10 and 20 day exposure to cigarette smoke . In adult life (6 months of age) following a 20 day postnatal exposure to cigarette smoke increased serum levels of corticosterone and prolactin were observed. Postnatal exposure to cigarette smoke produced increased CA utilization in the MPZ of the ME as well as a reduction in CA utilization in the PA 24 h after a 20 day exposure to cigarette smoke. These changes were not present in adult life.
The present results demonstrate that pre- and postnatal treatment with nicotine produces permanent activation CA nerve terminal systems of the external layer of the ME, mainly in the female rat.
Postnatal exposure to cigarette smoke produces hypersecretion of prolactin and corticosterone in the adult male rat in which hypothalamic CA nerve terminal systems seem not to play a major mediating role.
Taken together pre- and postnatal treatment with nicotine and postnatal exposure to cigarette smoke seem to produce functional consequences for neuroendocrine regulation as well as sex-specific responses in CA nerve terminal systems involved in food and water intake and in the response to stress in the male versus the female rat. A nicotine-induced disturbance in brain and pituitary cell replication and differantiation may underlie the permanent alterations observed.

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34 The effect of chronic nicotine treatment on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced degeneration of nigrostriatal dopamine neurons in the black mouse.

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Previous experiments have shown protective effects of chronic nicotine treatment against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced (MPTP) lesion of the mesostriatal dopamine (DA) system in the male C57Bl/6 mouse.

(-)-Nicotine was delivered for two weeks (0.125 mg·kg^{-1}·h^{-1}) via Alzet minipumps, which were implanted subcutaneously the day before MPTP (50 mg/kg, s.c.) was given. Starting immediately following the lesion (-)-nicotine (0.5 mg·kg^{-1}, i.p.) was given in four injections with a 30-minute interval. The immunohistochemical analysis applying the avidin-biotin immunostaining procedure, after incubation of the striatal cryotome sections with a polyclonal tyrosine hydroxylase (TH) anti-body, showed a counteraction of the MPTP-induced decrease of the total area of TH immunoreactive nerve terminals. In analogy, determinations of endogenous dopamine levels in the substantia nigra using high-performance liquid chromatography (HPLC) with electrochemical detection showed partial restoration of the MPTP-induced disappearance of DA stores.

Similar experiments giving six different doses (0.037mg·kg^{-1}·h^{-1} to 3 mg·kg^{-1}·h^{-1}) of (-)-nicotine for two weeks via Alzet Minipumps after MPTP lesions (40 mg·kg^{-1}, sc) have been carried out. To have a clear dose-response protocol no intraperitoneal (-)-nicotine injections were performed after the lesion in these experiments, where TH immunohistochemistry in combination with biochemical determinations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC were applied in analysis of the substantia nigra and the neostriatum.

Biochemical analysis of the neostriatum demonstrated a dose-dependent increase of the MPTP-induced decrease of the DA stores (DA levels - after MPTP alone : 59$\pm$7 %, after MPTP + nicotine 3 mg·kg^{-1}·h^{-1} : 28$\pm$3%). Applying the Jonckheere-Terpstra test DA ($p<0.01$), HVA ($p<0.01$) and DOPAC ($p<0.05$) showed a dose-related decrease with increased doses of (-)-nicotine in the MPTP lesioned animals. Also, in the dose 0.11 mg·kg^{-1}·h^{-1} nicotine produced an additional increment of the turnover increase found after MPTP alone. Similarly, the MPTP-induced decreases of TH immunoreactive nerve cell bodies, dendrites and terminals in the nigro-striatal system were enhanced with increased doses of (-)-nicotine.

In summary, it seems as if in the early experiments the (-)-nicotine injections immediately following the MPTP-lesion were necessary to partially protect against the MPTP-induced degeneration of the mesostriatal DA system probably due to the acute DA releasing action of nicotine counteracting MPTP uptake via the DA membrane pump. In contrast, in absence of such a protocol an enhanced degeneration of the DA neurons was found, which may be related to an enhancement of the DA turnover. Thus, the previously reported reduction of DA activity in mechanically lesioned DA neurons after nicotine treatment is not obtained. This difference between mechanical and neurotoxin-induced lesions of the meso-striatal dopamine system may be related to e.g. a membrane damage produced by MPTP.

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35 The effect of intra-accumbens nomiphensine on nicotine-induced dopamine secretion from the nucleus accumbens - a microdialysis study

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Previous studies suggest that nicotine preferentially stimulates mesolimbic dopamine (DA) systems and that the effect mediates the locomotor stimulant properties of the alkaloid (2,3). In our laboratory, the acute administration of nicotine has been shown to cause hyperlocomotion and a significant elevation in the extracellular concentration of the major metabolite of DA, dihydroxyphenylacetic acid (DOPAC), in the nucleus accumbens (NAc) (1). However, in these experiments, we failed to detect significant increases in extracellular NAc dopamine (DA). The present study investigated the possibility that blockade of the DA uptake mechanism would reveal the effects of acute nicotine on DA secretion in the NAc. Male Sprague-Dawley rats were anaesthetised with avertin and dialysis loops implanted stereotaxically in the NAc. 24 hours later, the probes were dialysed with a Ringer solution with or without nomiphensine (10μM) at a rate of 1.7μl/min. Nomiphensine, added to the Ringer solution, caused a four fold increase (F treatment (1,7) = 46.0, $p < 0.001$) in the basal extracellular levels of DA in the NAc (Fig 1a). Under these circumstances, the acute, subcutaneous injection of nicotine (0.4mg/kg) resulted in increases in DA in the dialysate (Fig 1b) which were significant when compared with saline-treated controls (F treatment x time (6,48) = 6.32, $p < 0.01$) and animals treated acutely with nicotine (F treatment x time (6,60) = 2.85, $p<0.05$) but with no nomiphensine in the probe. This concentration of nomiphensine had no significant effect on the extracellular concentration of DOPAC nor did it affect the increase in DOPAC (F treatment x time (6,48) = 6.48, $p<0.01$) observed in response to acute nicotine administration. In conclusion, under the conditions used in our laboratory, NAc DA secretion evoked by acute nicotine, is only detected unequivocally when the efficiency of the DA uptake mechanism is impaired by the inclusion of an uptake blocking drug in the perfusate.

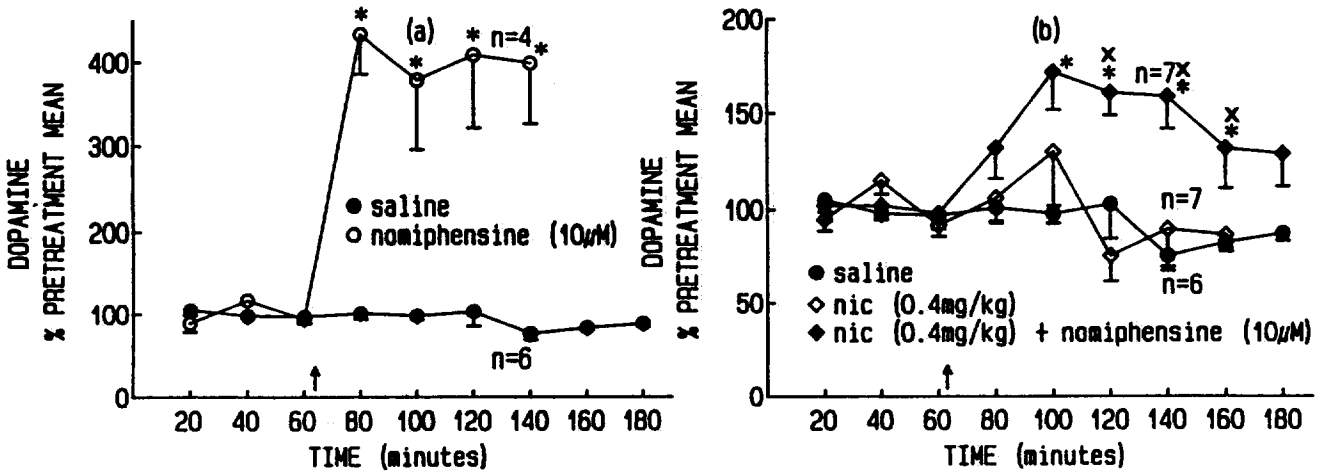

FIGURE 1 DA release in the NAc in response to the various treatments applied at the time indicated by the arrow. *p<0.05 cf saline, xp<0.05 cf nic.

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36 Effects of nicotine and nicotine withdrawal on local cerebral glucose utilization

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In previous studies we have investigated the effect of an acute (1) and a 2 weeks' (2) chronic infusion of nicotine on local cerebral glucose utilization (LCGU). LCGU is accepted to serve as an indicator of the local functional activity in the brain and was measured in awake rats in the previous studies as well as in the present investigation using the quantitative 2-deoxy D-(1-^{14}C) glucose method of Sokoloff et al (3). The present investigation was designed on the basis of two previous findings: 1. Chronic infusion of nicotine results in an increase in nicotine binding sites in the brain. This has been found by several groups. 2. Chronic infusion of nicotine, when compared to an acute infusion, induces partly congruent and partly divergent effects on LCGU in different brain structures (2). This led us to the question, whether a withdrawal of nicotine after a 2 weeks'exposure would restore the brain function (LCGU) to the preinfusion values within one day and whether an acute infusion after one day's withdrawal would influence LCGU. The main results of these studies are: a one day withdrawal of nicotine after a 2 weeks' chronic infusion restores LCGU in several brain structures: a pattern of LCGU which is close to control conditions is obtained. An acute infusion of nicotine after a one day withdrawal of a chronic infusion induces distinct increases in LCGU of several structures which had also been activated during an acute infusion of nicotine in otherwise untreated rats (no chronic infusion). The data indicate that the main effects of chronic nicotine infusion on cerebral function have disappeared after one day of nicotine withdrawal and that an acute load of nicotine in such nicotine-withdrawal rats has effects on LCGU which are rather close to those found in previously untreated rats.

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37 PRELIMINARY STUDIES ON THE IN VIVO DESENSITIZATION OF CENTRAL NICOTINIC RECEPTORS BY (-)-NICOTINE.

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Evidence for desensitization of the nicotinic receptor via nicotine has been suggested from two recent studies in this laboratory. The first study utilized a standard two-lever operant drug discrimination procedure. Rats were trained to discriminate 0.4 mg/kg (-)-nicotine from saline on a VI-15 second schedule of food reinforcement. When nicotine administration was preceded by an additional 0.4 mg/kg dose of nicotine, responding on the drug-appropriate lever was decreased in a sub-population of rats. The peak effect of the desensitization varied for individual rats and ranged from 15 minutes to 3 hours.

The second study examined the ability of (-)-nicotine to protect cholinergic receptors from an acetylcholinergic neurotoxin, AF64A (Ethylcholine aziridinium ion). The basic hypothesis is that nicotine might spare cholinergic receptors from AF64A via a desensitization of these neurons presynaptically. Rats were trained to perform a short-term memory task in an eight arm radial maze. Following training, rats were implanted with 14 day Alzet osmotic mini pumps containing (-)-nicotine (1.5 mg/day) or saline. Seven days after pump implantation, rats were injected intraventricularly (ivt) with 6 ng AF64A or with distilled water. All animals were tested for short-term memory deficits seven days after ivt injections. Animals receiving distilled water displayed no memory deficits, while those receiving AF64A following pre-treatment with saline displayed significant deficits. Animals infused with nicotine prior to receiving AF64A showed moderate memory deficits, suggesting that nicotine may have partially desensitized acetylcholinergic receptors, thus preserving some short-term memory ability. Further studies in this laboratory will attempt to determine the possible mechanisms of desensitization of nicotinic receptors by nicotine.

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38 Cellular Localization and Functional Properties of Nicotinic Receptors on Cultured Cortical Neurons from Rat Brain.

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Our laboratory previously identified putative nicotinic receptor sites in primary monolayer cultures of cortical neurons isolated from fetal rat brain (*J. Pharmacol. Exp. Ther.* 246: 409-416 (1988)). The equilibrium and kinetic binding properties of these sites, as well as their pharmacological specificity, are essentially the same as those determined for high affinity receptors in adult rat brain tissue. Methods have now been developed to localize these sites at the cellular level and to define their functional properties. We have found that anti-idiotypic antibodies against anti-nicotine antibodies are potent competitive inhibitors of [^{3}H]-nicotine binding to high affinity sites on the cells. These antibodies represent structural analogs of nicotine which have been shown to recognize neuronal nicotinic receptors (Bjercke, R.J. and J.J. Langone, *Biochem. Biophys. Res. Commun.* 162: 1085-92 (1989)). Consequently, we have used them to identify and characterize populations of receptor-bearing neurons by means of indirect immunofluorescence. We also have begun to explore the functionality of these receptor sites, using the ion-specific fluorescent probe fura-2 in conjunction with light microscopic image analysis techniques to measure ligand-evoked changes in intracellular calcium. The results indicate that the nicotine binding sites associated with these cells are functional nicotinic cholinergic receptors.

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39 THE NOVEL POLYPEPTIDE THYMOPOIETIN POTENTLY AND SPECIFICALLY INTERACTS AT THE NICOTINIC α-BUNGAROTOXIN RECEPTOR POPULATION IN NERVOUS TISSUE

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Evidence suggests that there exists at least two populations of nicotinic receptors in nervous tissue. These include the nicotinic acetylcholine receptor (nAchR) which binds monoclonal antibodies against the nicotinic receptor and which also binds [^{3}H]nicotine with high affinity but not α-bungarotoxin (α-BGT). The other site is the α-BGT site, which binds the α-toxin with high affinity and nicotinic receptor ligands with a lower affinity. With regard to function, the nAchR appears to be involved in mediating synaptic events, while the role of the nicotinic α-BGT site in the nervous system is not known. However, the observation that the toxin binds with such specificity and selectivity to a site with the characteristics of a nicotinic receptor, infers that the site is of significance; it may have developed to mediated certain specialized functions related to the nicotinic system.

In addition to the idea that the α-BGT site may mediate functions distinct from those conventionally associated with the nicotinic receptor, it is also possible that the toxin binding site may be the receptor for other ligands, as well as Ach. Recent studies in our laboratory have focused on thymopoietin, a 49 amino acid polypeptide isolated from thymus, which is involved in immune mediated responses. Thymopoietin potently and specifically inhibited [^{125}I]α-BGT binding (IC50 = 3-10 nM) to brain membranes. Nicotinic receptor ligands also inhibited α-BGT binding at uM concentrations, in agreement with previous work. Since thymopoietin has been identified in CNS tissue, these results suggest that the nicotinic α-BGT receptor population in brain can be affected by two distinct classes of agents, the polypeptide thymopoietin and nicotinic receptor ligands.

Studies were done to determine whether thymopoietin could regulate the nicotinic α-BGT receptor population. Chromaffin cells in culture, which are of neural crest origin, were used for this purpose since the α-BGT sites in these cells can readily be altered. Long term exposure (2 to 7 days) of the cells in culture to thymopoietin resulted in an increase in the number of α-BGT sites without affecting receptor affinity. Interestingly, the thymopoietin induced increase in the receptors could be reversed by exposure of the cells to nicotine. This finding suggests that the sites up-regulated by the polypeptide still retain nicotinic characteristics. It thus appears that the α-BGT site not only interacts with, but can also be regulated by, both nicotinic cholinergic ligands and the thymic polypeptide.

Experiments to assess possible functional consequences of thymopoietin in nervous tissue showed that thymopoietin enhanced process formation in a neuronal cell line (PC 12) in culture. These results could suggest that thymopoietin represents a trophic factor for neuronal cells, possibly interacting at the nicotinic α-BGT site. Experiments are currently in progress to determine whether nicotinic receptor ligands modulate the thymopoietin induced neurite extension in the cells in culture.

To conclude, the present work indicates that thymopoietin, as well as nicotinic receptor ligands, may be endogenous ligands for the α-BGT site. Studies to investigate the interaction between nicotine and thymopoietin at the α-toxin binding site in neuronal tissues are currently in progress

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40 NICOTINE INCREASES THE FIRING RATE OF VENTRAL TEGMENTAL AREA NEURONS *IN VITRO*

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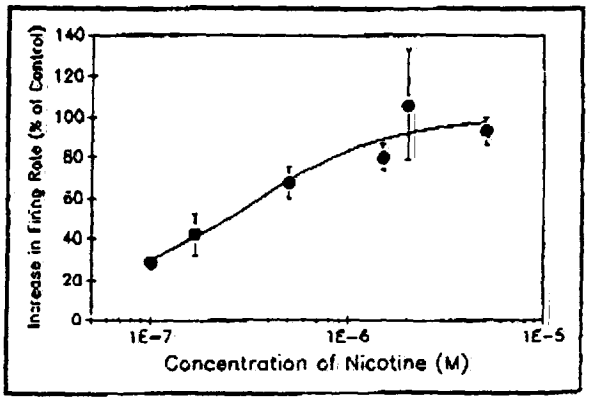
The ventral tegmental area of Tsai (VTA) contains a high concentration of dopamine-containing neurons, and has been implicated in the rewarding properties of drugs of abuse. Central dopamine systems, especially the mesolimbic and mesocortical pathways emerging from the VTA, have been shown to be important in the mediation of the rewarding effects of cocaine, amphetamine, opiates, and alcohol. Recent studies also suggest that mesencephalic dopamine neurons play an important role in the rewarding properties of nicotine. The action of drugs on VTA neurons can be studied with electrophysiological techniques *in vivo* but these studies are subject to a variety of limitations. Using a brain slice preparation of the VTA, neurons can be studied in a controlled environment: known concentrations of drugs can be applied, and the ionic and chemical environment outside of the slice can be precisely manipulated. I have used the brain slice technique to study the effects of nicotine on neuronal activity in a brain slice preparation.

Sprague-Dawley rats (male, 75-125 gm) were used in this study. For each experiment, a rat was sacrificed, its brain removed from the cranium, a block of tissue containing the VTA was prepared on ice, and a 400 μm coronal slice was obtained. This slice was placed in a recording chamber, and was superfused with artificial cerebrospinal fluid at a rate of 2 ml/min. Drugs were added to the superfusion medium via calibrated syringe pumps. Extracellular recordings of spontaneous firing rate over 10 sec intervals was recorded on a chart recorder; firing rate information was also recorded on an IBM-PC-based system for off-line analysis. Units were studied which conformed to electrophysiological parameters established for putative dopamine neurons (Brodie and Dunwiddie, *Brain Research* 425: 106-113, 1987).

Nicotine potently increased the firing rate of all neurons studied. Firing rate was increased up to 300% of basal firing rate. Increases in firing rate were apparent with the administration of concentrations of nicotine as low as 50 nM. With the administration of concentrations of nicotine up to 10 μM, desensitization to the excitatory effects of nicotine was not observed. The concentration-response curve for concentrations from 100 nM to 10 μM yielded an EC50 of about 250 nM. The excitatory effect of nicotine was completely blocked by hexamethonium, but was not altered by atropine. Acetylcholine also increased the firing of VTA neurons; this excitation was potentiated by a low concentration (200 nM) of physostigmine. Higher concentrations of physostigmine (2-10 μM) alone produced excitation, suggesting a tonic release of ACh in this brain slice preparation. The excitatory action of acetylcholine is not completely blocked by hexamethonium, but is blocked by a combination of atropine and hexamethonium, suggesting that both muscarinic and nicotinic receptors are found on VTA neurons.



These studies demonstrate that neurons of the VTA studies *in vitro* are activated by low concentrations of nicotine. Calabresi, *et al.* (*Brit. J. Pharmacol.* 98:135-140, 1989) have recently reported marked desensitization to the excitatory effects of 100 μM nicotine on VTA neurons in a brain slice preparation. By contrast, little or no desensitization was observed in the present studies with concentrations of 100 nM to 10 μM nicotine; therefore, it is possible that desensitization mechanisms are only triggered by very high concentrations of nicotine. Alternatively, only a portion of the nicotine-induced excitation of VTA neurons may be subject to desensitization, and this portion may not be as pronounced at lower concentrations of nicotine as at higher ones. Using concentrations below 10 μM, nicotine excites VTA neurons in a reversible, concentration-dependent manner.

The VTA slice preparation may become a useful tool for the study of agents which can alter the action of nicotine on central neural pathways involved in reward. The abuse of nicotine-containing agents may stem from the effects of nicotine on neurons of the VTA. Through the use of model systems like the VTA slice preparation, pharmacotherapy useful in smoking cessation treatment programs may be developed.

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41 BEHAVIORAL INTERACTION BETWEEN NICOTINE AND CAFFEINE: EFFECTS OF NICOTINE, CAFFEINE, AND THEIR COMBINATION ON LOCOMOTOR ACTIVITY

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The effects of the central nervous system stimulants, caffeine and nicotine, on spontaneous tunnel locomotion were determined in rats. In a first experiment, rats were daily injected for 10 days with nicotine (0.2mg/kg) or saline. During the next two days, they were exposed to a tunnel maze for two 6-min trials. On the third day, locomotor activity was measured (30-min trial) in the tunnel maze 15 min after subcatenous injection of saline, nicotine (0.4mg/kg), caffeine (8mg/kg), or nicotine and caffeine in combination. Acute exposure to nicotine decreased locomotor activity in nicotine naive rats, and this was antagonized by caffeine given in combination. Chronic nicotine exposure promoted the development of tolerance to the behavioral depression. No significant effect on locomotor activity was observed after caffeine administration.

In a second experiment, rats were daily exposed to the tunnel maze (6-min trials) 15 min after injection of saline or nicotine (0.2mg/kg). All rats were then sequentially exposed to caffeine dose of 4/8/16/32mg/kg. Activity levels in rats tested with saline declined over the sessions whereas intersession habituation was not observed under nicotine condition resulting in a significant difference of activity levels between the two groups. The highest dose of caffeine (32mg/kg) reduced the activity produced by nicotine in nicotine-tolerant rats whereas caffeine at low dose (4/8/16mg/kg) increased locomotor activity in saline-treated rats.

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42 Effects of pre- and postnatal injections of "smoking doses" of nicotine, or vehicle alone, on the maternal behavior and second generation adult behavior of Roman high- and low-avoidance rats

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RHA/Verh and RLA/Verh rats are selected and bred for the rapid versus non-acquisition of two-way, active avoidance behavior, respectively. Based also on other behavioral, neurochemical and hormonal evidence, the RLA/Verh line is considered to be the more anxious of the two. Differences in the sensitivity to nicotine between the two lines have long been recognized in connection with locomotor behavioral tasks. The present studies were intended to expand this knowledge on a practical level, being concerned with the interactional effects of pre- and postnatal nicotine administration and stress on two generations of maternal behavior of females, as well as the adult behavior of males derived from the exposed litters.

Female rats of both lines were either undisturbed (controls), or injected 3-4 times per night with physiological saline (stressor alone) or with 0.3 mg/kg nicotine, i.p., starting 2 weeks before giving birth and continuing until 2 weeks after giving birth. Maternal behavior observations were made during the daytime, using a time-sampling method. Effects of the stressor injections alone were seen almost exclusively in RLA/Verh mothers; e.g. a doubling of their active/passive position ratio, an increase of 50 percent in sleeping while separated from the pups and a 20 percent reduction in time spent in the nest. Remarkably, none of these changes occurred in the rats receiving nicotine injections as compared to control (undisturbed) mothers, indicating that nicotine attenuated the stress response elicited by the injections of vehicle alone. Even more remarkably, when the females derived from these litters matured and bore their own young, the RLA/Verh rats whose mothers had been injected with saline (i.e. the rats which had been indirectly stressed) showed the same changes in maternal behavior that their mothers had shown. Once again, however, these changes were not seen in the maternal behavior of RLA/Verh rats whose mothers had received the nicotine injections.

Studies were also conducted utilizing adult males derived from the first generation litters, and the observation was made that RHA/Verh rats which had been exposed to nicotine in utero and postnatally showed a retarded acquisition of the two-way, active avoidance response, when compared to controls. As associations had been previously suggested to exist between a) a role for the hippocampus and the behavioral effects of nicotine in these rat lines, and b) the extent of the hippocampal infrapyramidal mossy fiber projection and a predisposition to acquire the avoidance response in RHA/-Verh (versus RLA/Verh) rats, the hippocampii of the RHA/Verh rats involved were examined microscopically, using Timm-stain and computer analysis. It was found that pre- and postnatal exposure to nicotine indeed altered the size of the infrapyramidal mossy fiber projection, reducing it to a marginally significant degree. As this change was in the opposite direction to that which would have been expected based on the shuttle box results, however, it was concluded that the behavioral effects of nicotine seen were most likely mediated elsewhere, one possibility being the mesolimbic/nigrostriatal dopaminergic pathways, where profound differences in reactivity have been previously found between RHA/Verh and RLA/Verh rats, reflecting their divergent, inherent locomotor and emotional characteristics.

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43 Alterations in septohippocampal cholinergic receptors and related behavior after early exposure to nicotine.

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In previous investigations we established a mouse model for the study of drug neuroteratogenicity; phenobarbital was then selected as the model drug. The studies ascertained alterations in the septohippocampal cholinergic pathways related to prenatal phenobarbital-induced deficits in hippocampal-dependent behaviors. There were increases in the B_{max} of muscarinic receptors and their second messenger, inositol phosphate, after early exposure to phenobarbital, while almost no presynaptic cholinergic alterations were found. Concomitant deficits were demonstrated in the hippocampus-related eight-arm maze behavior. Consequently, the behavioral deficits could be reversed by transplantation of cholinergic cells to the impaired hippocampus. Therefore, it is now becoming feasible to apply this model on the more commonly abused nicotine. Mice were exposed to nicotine prenatally by injecting the mother 1.5 mg/kg nicotine s.c. once daily on gestation days 9-18 (PreN mice), or neonatally, by daily s.c. injections of 1.5 mg/kg nicotine to the pups on days 2 to 21 (NeoN mice). On age 50 days, the mice were tested in the eight-arm maze and their hippocampi were assayed for muscarinic receptor binding using ^{3}H-QNB as a ligand. PreN and NeoN mice made 28% more errors in the maze than control before reaching criterion ($p < 0.01$). Hippocampal muscarinic receptors B_{max} of controls was 1.12±0.21 pmol/mg protein (Mean±SEM). The score for PreN was 1.77±0.02 and for NeoN mice 2.00±0.06; increases of 58% and 79% from control respectively ($p < 0.01$). On the other hand, K_D was unaffected by early nicotine exposure. Whether alterations can be demonstrated in the subsequent steps of the postsynaptic cholinergic transmission and, similarly to the phenobarbital model, the biochemical and behavioral deficits can be reversed by neural grafting, remains the subject of our current investigations.

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44 INFLUENCE OF NEONATAL NICOTINE EXPOSURE ON DEVELOPMENT OF NICOTINIC RECEPTOR SUBTYPES AND BEHAVIOUR IN ADULT MICE

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The specific binding sites for ^{3}H-nicotine in mice brain are detectable during late prenatal period and gradually increase during postnatal period from day of birth until about 28 days of age, when they reach adult level(1). The competition curves of ^{3}H-nicotine/(-)nicotine in mice cortex of 1 day and 5 days of age are best fitted to an one-site model, and in adult mice cortex, to a two-site model (1). Subchronic treatment of rat with nicotine significantly increases the number of high affinity nicotinic binding sites with a parallel increase in the proportion of high affinity binding sites compared to low affinity binding sites in rat cortex (2). The present study intended to investigate, by means of receptor binding assay and behavioural test, the influence of neonatal nicotine exposure on development of nicotinic receptor subtypes and behaviour in adult mice.

Male NMRT mice received nicotine (66 µg base/kg) twice a day (8 am. and 5 pm.) between 10-16 postnatal day. The control group received saline in the same way. Some of the mice were killed at 17 days of age, and their cortices were used for binding assay. The others were kept until adult age of 4 months, when they after behavioural test were killed and their cortices were used for binding assay. The displacement curves for ^{3}H-nicotine/(-)nicotine in control cortex of 17 days mice were best fitted to a two-site model with affinity constants of 0.38 nM (K_H) and 14 nM (K_L), respectively. A two-site model fit was also observed in control adult cortex with a K_H 3.6 nM and a K_L 1.2 µM thus about ten and hundred times higher than those for 17 days mice, respectively. Interestingly, the ^{3}H-nicotine/(-)nicotine displacement curves of nicotine treated 17 days and adult mice were best fitted to an one-site model. The affinity constant (K_D) was 2.1 nM for 17 days mice and 3.0 nM for adult. There was no difference in Bmax between nicotine treated and control adult mice. Nicotine induced behaviour was studied by using two different doses of nicotine; 40 and 80 µg nicotine base/kg body weight. The result from locomotion, rearing times and total activity showed that mice receiving nicotine between 10-16 postnatal day displayed a hypoactive condition, whereas mice receiving saline displayed a hyperactive condition. In conclusion, neonatal treatment with nicotine caused a change in the nicotinic receptors in adult animals with a loss in low affinity binding sites; it also resulted in an opposite behavioural effect of nicotine in neonatal nicotine treated animals compared to naïve animals.

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45

Rearing and learning behaviors of rats after long-term administration of nicotine

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Behavioral effects for rats after long-term administration of nicotine were investigated, especially on rearing or watching attitude and learning Behaviors. Administration of nicotine for rat was performed by drinking the tap water which contain 0,01% nicotine distilled newly (114°C,35mHg). The activities of locomotion, rearing were measured by a behavior analyzer equipped with ten beams infrared light in the X-Y axis. T maze learning for rats (motivation; 80% reduction of weight by diet) were performed by judgment of left or right entrance into traps on the way for goal.

Result and discussion: Nicotine administered rats (N) were compared with control (C; not treated by drug). N were slightly increased the activities of locomotion in night, but their rearing and watching attitude were more intensive than C.

The sixth trials of learning of rats in a day time were tested during 5 days. N were reinforced, because the latency and errors number in learning were more shorter and smaller than C. Scopolamine (40mg/Kg) effects on post-learning after 4 days for C, but not N. The reverse learning were more influential in a time course of training for both N and C.

Nicotine levels in plasma of N were 0,14-0,24 μM after 2 weeks drink, cotinine levels were higher than that of nicotine after 5 days administration of nicotine in tap water. The increasing of rearing behavior of N was matched at time of rising of cotinine excretion of N after nicotine administration, also learning of N were reinforced.

It seems that N were acquired a reverse tolerance for nicotine on behavioral effects in these experiments after long-term administration.

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46 COMBINED EFFECT OF NICOTINE AND TOBACCO MOSAIC VIRUS ON DOPAMINE RECEPTORS

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Nicotine[1] and Tobacco Mosaic Virus (TMV)[2] are present in every tobacco product. Studies investigating the role of nicotine have suggested its effect in the maintenance of smoking and tobacco consumption behaviour. It also has a mild stimulatory action on the central nervous system. Besides this, TMV which is although non-pathogenic and does not multiply in the animal host has been found to effect the synaptic mechanism[3]. In order to study the single and combined action of nicotine and TMV on dopamine receptors, nicotine (1 mg/Kg) and TMV (10 ug/animal) i.v. were given to rats for 2 weeks. Receptor binding studies carried out on brain synaptosomes using 3H-spiroperidol indicated highest binding in the group of rats which received combined doses of nicotine and TMV. Behavioural studies were also performed to measure amphetamine (2.5 mg/Kg, i.p.) induced stereotypy at 10 and 30 min. Within 10 min amphetamine produced increased movements and stereotypy in normal and nicotine treated rats only, but this behaviour was observed in the group receiving TMV alone and nicotine + TMV only after a lapse of 30 min. This delayed onset of stereotype behaviour in the latter groups suggest disfunctioning of dopaninergic neurons due to nicotine and TMV. Increased dopamine receptor binding in conjugation with delayed amphetamine response suggest for synergestic action of nicotine with TMV in the synaptic process.

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47 The Influence of Nicotine on CNS Arousal during the Menstrual Cycle

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The Influence of Nicotine on CNS Arousal during the Menstrual Cycle.

Critical Flicker Fusion Threshold (CFFT), used as a measure of cortical activation, and prospective reports of menstrual distress were recorded in 47 women (30 non-smokers, 9 light smokers and 8 heavy smokers) at regular intervals during the menstrual cycle. A significant linear trend was found within the pooled CFFT data (P 0.0004) seen as a gradual increase in cortical arousal across the menstrual cycle. The division of data into smoking groups indicated that this pattern was maintained in both non-smokers and light smokers but not among the heavy smokers who showed significantly lower CFFTs (P 0.05). A possible depressant effect of nicotine is discussed and the relationship of the present results to changes in smoking patterns during the menstrual cycle considered.

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48 SEPARATE AND COMBINED EFFECTS OF THE SOCIAL DRUGS ON PSYCHOMOTOR PERFORMANCE

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Ten subjects (five smokers and five non-smokers) performed a choice reaction time task (CRT), a compensatory tracking task (CTT), a short-term memory task (STM) and were tested for their critical flicker fusion threshold (CFF) at set points over four hours after administration of each possible combination of nicotine (2mg gum or placebo), caffeine (250mg capsule or placebo) and alcohol (30g or placebo). CTT performance was significantly improved whenever nicotine was included in the drug combination or when caffeine was administered alone, but impaired by alcohol alone. STM retrieval was signifcantly facilitated by either nicotine or caffeine alone and significantly disrupted by the combination of alcohol and nicotine. The motor response component within the CRT task was facilitated by both nicotine and caffeine. CFF results lay in predicted directions but were not significantly affected by any drug combination.

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49 EFFECTS OF NICOTINE GUM ON SHORT-TERM MEMORY

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An experiment was conducted to investigate the effects of nicotine on memory function independent of any performance decrements associated with tobacco deprivation. 20 subjects (10 non-smokers and 10 smokers who had been allowed to smoke normally until testing) attended the laboratory at their "preferred nicotine level" and completed a short-term memory task (memory scanning) at set points for 4 hours after the administration of 2mg or 0mg (placebo) nicotine polacrilex gum. The results suggest that nicotine enhanced memory reaction time performance (P 0.01) when subjects were probed for information already present in short-term memory (correct positive responses) but had no effect on reaction time when the information was absent from memory (correct negative responses). It is hypothesised that changes in electrocortical arousal after the administration of nicotine facilitate the processing of stimulus information in short-term memory.

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50 Nicotine polacrilex gum and sustained attention

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The effects of nicotine polacrilex gum upon sustained attention were investigated in three studies. Each study was double-blind and placebo controlled, with drugs administered in a counterbalanced order. The subject groups comprised 16 smokers, different in each study, who had abstained from smoking for 12 hours prior to testing. Sustained attention was assessed using the Wesnes and Warburton rapid visual information processing (RVIP) task.
In the first study, nicotine increased the number of target detections in a significant dose related manner ($p<.01$), with the greatest performance improvement under cigarette smoking (+2.2 targets), a smaller improvement under 4 mg gum (+1.9 targets), almost unchanged performance with 2 mg gum (+0.2 targets), and a comparative performance decrement under placebo (-2.2 targets). Response times showed a significant curvilinear function ($p<.01$), with improved times under 2 mg gum and 4 mg gum, while times under placebo and cigarette were close to baseline.
In the second study, the effects of nicotine were investigated upon a battery of 4 attention tasks: RVIP (as above), letter cancellation, Stroop, and a width of attention task. The RVIP task findings were similar to those found in study 1, with improved target detection under the nicotine conditions, and performance decrements under placebo. The main difference from study 1 was that target detections were improved to the greatest extent under 2 mg gum. RVIP response times again showed a curvilinear function, almost identical to that shown in the previous study. These findings, have implications for the Yerkes-Dodson inverted-U function hypothesised to underly the arousal-performance relationship. Slight differences in experimental design, leading to different durations of nicotine ingestion (necessitated by the longer test sessions in study two), may have contributed to the subtle RVIP performance differences between studies 1 and 2. Width of attention was not affected by nicotine, nor was Stroop task performance, but letter cancellation times were significantly faster under smoking. Overall, nicotine therefore improved sustained attention or 'vigilance', but did not affect the width of attention or attentional selectivity.
The third study compared 8 regular smokers (+15 cigarettes/day), with 8 non-regular smokers (-5 cigarettes/day), to investigate whether the degree of nicotine deprivation affected response to 4 mg nicotine gum. Regular smokers reported significantly higher ratings of nicotine 'craving' before testing ($p<.05$). RVIP target detections in the regular smokers increased under nicotine gum (+3.3 targets), but decreased under placebo gum (-1.1 targets), while in the non-regular smokers, target detection rates were similar under nicotine and placebo. Although the ANOVA group x drug interaction effect was not statistically significant ($P=0.13$, two-tail), the above finding is broadly in agreement with previous research which suggests that nicotine only improves performance in nicotine deprived subjects. Thus non-regular smokers, not being in a state of acute nicotine deprivation, showed little performance change following the nicotine gum.

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51 Nicotine deprivation and nicotine reinstatement: effects upon a brief sustained attention task

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Investigations into the effects of nicotine upon human performance, of necessity, use nicotine deprived subjects. However, since nicotine deprivation leads to impaired performance, the interpretation of any performance change following nicotine is open to question: does the improved performance reflect a genuine improvement above the normal baseline, or does it reflect the reinstatement of performance levels found prior to deprivation? In order to answer this question, the effects of nicotine deprivation, and of nicotine reinstatement, need to be assessed within the same study. This has not been undertaken before, but was the aim of the present study. A secondary aim was to assess whether nicotine deprivation would be evident using only a brief attentional task, since previous research demonstrating performance decrements following nicotine deprivation had used prolonged tasks of 1-6 hours duration.

Twenty female nurses, all regular smokers (+15 cigarettes/day), were assessed on a brief letter cancellation task. Each response sheet contained 105 letter targets (60 high frequency, 30 mid frequency, and 15 low frequency). Following training, subjects were tested on four successive days. Days one and four comprised baseline days. Then either on day 2 (half the subjects) or on day 3 (other half of subjects), the volunteers agreed to abstain from smoking before testing (12+ hours abstinence). On the other three days normal smoking was permitted up to 30 minutes before testing. The first test session was given prior to smoking. This was followed by one cigarette, then a second letter cancellation test.

Performance was significantly impaired by nicotine deprivation, when assessed both by response time ($p<.05$) and target detections ($p<.05$). There was no evidence of a speed accuracy tradeoff; the correlation between target detection change and response time change was near to zero. On the second test following the cigarette, the performance of the previously deprived subjects returned to baseline, while performance remained unchanged in the other conditions. Throughout the experiment, there was no evidence of performance differences between the high, mid and low frequency targets. Overall therefore, nicotine deprivation led to an impairment in sustained attention, while reinstatement of nicotine led to performance close to baseline. There was no evidence of changed attentional selectivity, either during nicotine deprivation, or nicotine reinstatement.

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52 Concentrations of Smoke Constituents as Related to Nicotine Yield, Daily Consumption, and Individual Smoking Behavior

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The concentration of smoke constituents in blood is influenced by cigarette yield, the number of cigarettes smoked per day (CPD), and the individual pattern of smoking. However, the importance of the different parameters is still unclear.

In a cross-sectional study (N=144) we investigated the influence of the standardized nicotine yield (0.1-1.2mg) of subjects' usual brand on individual smoking behavior (topography) and different biochemical measures of smoke uptake. Subjects smoked one cigarette in the laboratory with normal lip contact and free puffing. In addition, smoking conditions were partly standardized by the introduction of a cigarette holder, allowing the measurement of puff volumes (and precluding the closure of ventilation holes), and by a forced puff procedure consisting of 30 puffs (3 puffs on 10 half cut cigarettes) within a restricted time period (max.12min). These smoking conditions were combined in a two by two repeated measures design (natural/forced puffing; lip contact/holder). Before and immediately after smoking, tidal air and blood samples were collected for determination of CO and nicotine and cotinine concentration, respectively; the change in concentration from pre- to postsmoking is seen as uptake.

CO concentrations in tidal air are mostly influenced by CPD ($.23<r<.42$), whereas the influence of nicotine yield is only modest ($.15<r<.33$). Nicotine and cotinine concentrations in plasma are influenced both by CPD ($.20<r<.47$) and nicotine yield ($.29<r<.58$), the relationship between nicotine yield and concentration being stronger if the concentration is measured immediately after smoking or with a fixed delay after smoking. With respect to smoke uptake from a single smoking period, nicotine absorption is strongly related to nicotine yield ($.39<r<.56$), the relationship becoming stronger with both forced puffing and holder smoking. CO uptake is less related to nicotine yield; higher correlations were found for forced puffing ($r=.34$ vs. $r\approx.22$ for natural puffing). For smoke uptake measures further relationships with smoking behavior variables are shown.

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53 Nicotine Exposure and Puffing Behaviour in the Young Habitual Low-Yield Cigarette Smokers

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Nicotine exposure measured by urinary cotinine and puffing parameters in 36 normally smoking students was studied. Three groups formed according to their own cigarette brand preference were compared with each other. 18 of them had always smoked low-yield (L-Y smokers), 10 medium-yield (M-Y smokers) and 8 had recently changed to low-yield cigarettes (Switchers). The subjects smoked with no limits their own brand for the first week, the second week low-yield cigarettes (tar 5 mg, nicotine 0.4 mg) and the third week medium-yield cigarettes (tar 15, nicotine 0.9 mg). Day urine samples were collected during the two last days in the test weeks and cotinine concentrations were analysed. Puffing indices were recorded on the last day of every test week using a portable microcomputer assisted analyser with flowhead cigarette holder. The measurements made were the number of puffs, puff duration, puff interval, smoking time, puff volume per cigarette and total puff volume per day.

The table shows that urinary cotinine concentrations (UCot, µg/ml) in the groups are rather constant with different cigarette types but are clearly lowest in the L-Y smokers. Nicotine content of cigarettes causes a compensatory effect on puffing behaviour in every smoking group. However, the correlation between cotinine concentration and total puff volume per day (L/Day) was poor. Puff parameters reveal that compensation is caused by increases in the number of puffs, single puff volume, puff duration and total smoking time. Switchers smoked more cigarettes than the others. The compensatory effect with the number of cigarettes was small in all the groups.

SMOKER GROUP		CIGARETTE TYPE Own brand	Low-yield (mean ± SEM)	Medium-yield
L-Y Smokers:	UCot	1.12 ± 0.14	0.90 ± 0.11	1.23 ± 0.18
	puffvol/day	10.4 ± 2.2	12.2 ± 3.2	7.2 ± 1.7
Switchers:	UCot	1.45 ± 0.18	1.78 ± 0.35	1.64 + 0.25
	puffvol/day	11.7 ± 2.9	16.9 ± 3.8	9.1 ± 2.8
M-Y Smokers:	UCot	1.54 ± 0.24	1.33 ± 0.22	1.51 ± 0.23
	puffvol/day	7.0 ± 1.4	10.6 ± 2.5	7.1 ± 1.7

The results of urinary cotinine concentrations and the past smoking history of subjects suggest that if smoking is started with low-yield cigarettes it will take 5-10 years to reach the same nicotine exposure levels as in medium-yield smokers and switchers. Hence, the benefit probably gained with low-yield cigarettes is not long lasting due to the compensatory behaviour in nicotine intake, mainly caused by the tolerance to nicotine. Individual variation is remarkable. Cotinine levels and puffing behaviour of the switchers group show that the change to lower tar/nicotine yield cigarettes is useless because the smokers increase puffing intensity.

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54 LONG-TERM USE OF "LOW NICOTINE YIELD" CIGARETTES*

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This study examined the long-term use of cigarettes with reduced smoke yields of tar, nicotine and carbon monoxide. Participants for the study came from a heterogeneous population of volunteers (1025 men and 807 women) recruited from blood drives in Bergen County, N.J., between 1983 and 1986. A blood sample was obtained from each subject and a questionnaire was administered on smoking behavior. Blood samples were analyzed for nicotine and cotinine by radioimmunoassay (RIA) and for thiocyanate (SCN^-) and carboxyhemoglobin (COHb) by established analytical procedures.

Differences in the relative blood concentrations of tobacco smoke markers were not evident between the sexes when the data were normalized for daily nicotine availability. Comparison of nicotine, cotinine and gas phase constituents between subjects who smoked low-yield and high-yield cigarettes revealed that those persons who had switched to the low-yield products during their smoking years had levels of smoke metabolites which were equivalent to those found in users of high-yield products. A variety of smoking behaviors including increases in cigarettes smoked per day, depth of inhalation and smoking to a shorter butt length could contribute to these levels. Survey data collected with the blood sample queried such changes in smoking behaviors with changing to these low-yield products.

Finally, smokers who had continuously smoked low-yield cigarettes were found to absorb similar levels of nicotine as did smokers who used high-yield products. These findings suggest that smokers might titrate nicotine in a fairly constant manner despite the reported tar/nicotine yield of the product. In theory, while reduction of the tar and nicotine components in cigarette smoke could reduce the incidence of tobacco related diseases, mechanisms of compensation and possibly nicotine titration can result in higher circulating levels of tobacco toxins than would be expected to be found with use of low-yield products.

* For this study, we regarded low nicotine/tar cigarettes as those cigarettes that deliver less than 0.7 g of nicotine and less than 10 mg of "tar" under standardized FTC machine smoking conditions.

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Cotinine concentration in the serum of smokers within a representative population in Southern Germany measured in 1984/85 and 1987/88

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As part of the MONICA Study on cardiovascular risk factors conducted in Augsburg (1) serum cotinine levels were quantitated in self reported smokers and non-smokers during the examinations taking place in 1984-85 and in 1987-88. In this paper, we demonstrate in a subsample of the cohort (508 smokers) providing cotinine data at both time points, the percentage of smokers changing to cigarettes of different nicotine yields, the percentage of smokers changing number of cigarettes smoked as well as the dependance of serum cotinine levels on these parameters. Between the first examination and the second, only a small percentage of smokers reported a change of their cigarettes which moved them between low yield (<0.6 mg nicotine) and medium yield (0.7-1.0 mg nicotine) or between high yield (>1.0 mg nicotine) and medium yield cigarettes. None of the smokers reported more dramatic changes between low and high nicotine yield cigarettes. At both time points, the nicotine yield of the cigarettes did not exert a significant effect on serum cotinine levels. In contrast however, number of cigarettes smoked per day had a significant impact on serum cotinine levels. From further analysis of our data, we conclude that smokers can up or down regulate nicotine intake when they switch from their regular medium yield cigarette to cigarettes with high or low nicotine yield.

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56 EFFECT OF NICOTINE ON THE ELASTINOLYTIC ACTIVITY OF HUMAN LUNG TUMOR CELLS AND HUMAN ALVEOLAR MACROPHAGES

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An experimental system was established which allows the determination of cell associated elastinolytic activity. Cells were confronted with radioactive labeled elastin in culture microvessels in medium (RPMI 1640). After 72 h at 37 ^{o}C the elastinolytic activity could be calculated from the released radioactivity. For stimulation experiments nicotine was added directly to the incubation medium.

Proteolytic enzymes are thought to play a role in tumor cell invasion (1). Therefore two human tumor cell lines were used in order to study the effect of nicotine on the elastinolytic activity. HS-24 was established from a primary squamous cell carcinoma of the lung, SB-3 from a metastasis of a primary lung adenocarcinoma into the adrenal gland (2). In the case of HS-24 nicotine showed no effect on the elastinolytic activity in a concentration of 50 ng/ml. However, 100 ng/ml and 200 ng/ml had an stimulating effect. The effect was found to be less in the case of 200 ng/ml. A stimulating effect was also found when the tumor promotor 12-O-tetradecanoylphorbol-13-acetate (PMA) was added in a concentration of 5 x 10^{-9} M. In the case of SB-3 cells nicotine had no effect on the elastinolytic activity in the range betweeen 50 and 200 ng/ml. Also no stimulating effect was found when PMA was used.

Proteolytic enzymes are also thought to play a role in the pathogenesis of emphysema (3). Because of this the elastinolytic activity of alveolar macrophages (AMs) isolated from bronchoalveolar lavage fluid (BALF) of patients with and without emphysema was investigated. The existence of an emphysema was characterized by lung function values. Our first results indicated, that AMs isolated from BALF of patients with emphysema possess an higher elastinolytic activity compared to AMs of patients without emphysema. The addition of nicotine (100 ng/ml) had an stimulating effect on AMs from patients with clinical signs of emphysema, whereas AMs of patients with nonemphysematous lung diseases were less or not stimulated.

In summary our results point to the important role of cell associated elastinolytic enzymes in tumor invasion as well as in the development of lung emphysema.

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57 **THE ASSOCIATION BETWEEN SERUM COTININE, CIGARETTE SMOKING AND BLOOD PRESSURE: THE CARDIA STUDY**

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Several studies have noted that cigarette smokers, on average, have lower blood pressures than do non-smokers. While the acute effects of nicotine include a transitory rise in blood pressure and release of catecholamines, the reasons for overall lowering of blood pressure in smokers was unknown. Cotinine, the major blood metabolite of nicotine, had been considered to be pharmacologically inert in humans (Russell, 1980), although an early study in dogs had reported depressor effects after injection of cotinine (Borzelleca, 1962) More recent research in animals (Dominiak et al, 1985) and a cohort study in humans (Benowitz,1989) have documented the association of lower blood pressures with serum cotinine levels.

The Coronary Artery Risk Development in (Young) Adults (CARDIA) Program is a longitudinal epidemiological study of risk factors for cardiovascular disease in persons aged 18-30. From four clinical centers located around the United States, 5,115 persons were recruited into the study. The cohort was roughly balanced by age, sex, race (black and white) and years of education. Cigarette smoking behavior and exposure to environmental tobacco smoke was determined by questionnaire and validated by serum cotinine measurements. Cotinine was quantitated by radioimmunoassay, and blood pressures determined according to established methods.

Of the cohort of 4,992 persons with complete smoking and blood pressure data, 29% were self-reported smokers with cotinine levels consistent with smoking status. Analysis of the association between blood pressure and cotinine in the entire cohort revealed that serum cotinine was inversely correlated with diastolic blood pressure (DBP) in the four sex-race groups ($r = -.08$ to $-.15$) and was significantly correlated with systolic blood pressure (SBP) in white men only ($r = -.10$ to $p = .0005$). In multiple linear regression, controlling for age, BMI, alcohol, plasma cholesterol and ex-smoking, the same DBP and SBP associations persisted ($p<.0005$).

From these results, DBP was estimated to be 1.9 to 2.8 mmHg lower for an increase in cotinine corresponding to the average difference between a non-smoker and a moderate smoker (11-20 cigarettes/day). In 2,593 subjects with non-zero cotinine values, the inverse association with DBP was again observed for all race-sex groups and with SBP in white men.

These findings support the association of cotinine with lower blood pressure in smokers in a large, racially mixed cohort of young adults, and support the need to determine whether cotinine is responsible for lower blood pressure in smokers compared to non-smokers.

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58 Doppler flow measurements and smoking in pregnancy

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30 pregnant smokers were evaluated by Doppler ultrasound to assess the possible effect of smoking a cigarette of her commonly-used brand.

We used a duplex doppler scanner (ADR 5000, Kranzbühler) to evaluate three vessels (fetal aorta, umbilical artery, uteroplacental vessels). After smoking, maternal pulse and blood pressure were registered in three minute intervals. Additionally, we included continuous CTG monitoring.

The examination time varied between 27-52 minutes (mean: 37). All doppler and laboratory parameters were evaluated before smoking (including nicotine and cotinine levels). Following that, the patient smoked a cigarette within about 5 minutes. Immediately afterwards continuous registration was started. 30 minutes later a second blood sample was taken for laboratory analysis.

Mean nicotine-/cotinine serum levels before smoking were 5.9/112 ng/ml, 30 minutes later 9.8/125 ng/ml. In pregnancies with no previous pathology (N=27) cigarette smoking had no discernible negative influences on doppler parameters in fetal and maternal placental vessels. With reluctancy (N=3 cases) this was also true for complicated pregnancies .

Fetal heart rate in cases with uncomplicated pregnancy were found to be unchanged during the 30 minutes. In one case with hypertension in pregnancy and in one diabetic mother there was an increase in fetal heart rate during the first 9 minutes following maternal smoking.

We conclude that there are no acute changes which can be depicted with doppler flow parameters after cigarette smoking in uncomplicated pregnancies.

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59 Cigarette smoking and platelets

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To assess the long-term effects of smoking on platelet-vessel wall interaction and platelet function we studied ten identical male twin pairs (mean age 40 years, range 31-53) discordant for smoking (average 18 cigarettes/day, range 5-32) for over 20 years.

In a duplex-ultrasound study the smoking cotwins had significant signs of carotid atherosclerosis, the area of plaques being 6.5 mm^2 vs 1.9 mm^2 of nonsmoking ones (mean paired difference 0.9-8.3, 95% confidence interval). The urinary excretion of 2,3-dinor metabolites of thromboxane A_2 (Tx-M) was increased in smokers in each pair: 480±68 (mean ±SEM) vs. 263±27 pg/creatinine mg in nonsmokers. Also, PGI-M was increased in smoking cotwins, 132±20 vs. 100±14 pg/creatinine mg in nonsmokers. The ratio between Tx-M and PGI-M was higher in smokers. Both packyears (r=0.83) and cotinine excretion (r=0.67) correlated with the urinary Tx-M in smokers.

Plasma fibrinogen was 3.0±0.1 g/l for nonsmokers and 3.3±0.2 g/l for smokers and hematocrit was 45±1 and 47±1, respectively. Added up these values imply that smoking elevates plasma viscosity ($p<0.01$). Platelet aggregation in vitro, and subsequent release of TxB_2 and serotonin did not differ at rest. However, after submaximal exercise smokers' platelets turned markedly refractory to agonist stimulation and the production of serum TxB_2 was reduced. Simultaneously, the α_2-adrenoceptor binding and prostacyclin responsiveness of adrenaline-stimulated platelets remained unaltered.

Our findings with these unique study subjects show that long-term cigarette smoking promotes progression of atherosclerosis and signs of increased platelet-vessel wall interaction. The reduced platelet responsiveness after exercise supports in vivo activation of platelets. In favor of enhanced risk of thrombogenesis are the increased levels of the contributors of plasma viscosity. Our study provides evidence that smoking-induced effects on platelets are both direct and indirect via enhanced arterial plaque formation.

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60 ABSTENTION FROM SMOKING MAY INCREASE THE RISK OF ULCERATIVE COLITIS BY ENHANCING EICOSANOID PRODUCTION

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Former smokers are at a greater risk of ulcerative colitis (UC) than either never smokers or current smokers. Analysis of available data showed that current smokers had a 60% reduction in risk of UC, while former smokers have a 2.1 fold increase in risk compared to never smokers. The onset of symptoms following smoking cessation varies for one month to several years, with a mean time lapse of 5.7 years. Cigarette smoking before the onset of the disease may also affect the clinical course of condition, in that former smokers require more hospitalisation, and more frequently undergo colectomy than never smokers. This suggests a causal relationship between former smoking habit and severity of the disease, with the effect being dependent on the magnitude of tobacco consumption.

Previous studies from this laboratory have shown that colonic mucus production varies relative to smoking history, both in controls and patients with UC. The eicosanoids, particularly prostaglandin E_2 and leukoriene B_4, have been shown to be elevated in the peripheral circulation and at the mucosal surface of patients with ulcerative colitis, particularly when the disease in its active phase. The eicosanoids are important mediators of the immune response, being responsible for purpetuating the cellular infiltrate into the mucosa. The aim of the present study was to determine to effect of smoking history on in vitro eicosanoid production by colonic tissue for patients with ulcerative colitis and controls.

Pinch biopsies from the colonic mucosa were removed from 39 patients with UC, of whom 25 were never smokers , 8 ex-smokers for 10 years or less, and 6 ex-smokers for more than ten years. The control group, which consisted mainly of patients with the irritable bowel syndrome and diverticular disease, contained 25 never smokers, 17 (< 10 years) and 23 (> 10 years) ex-smokers. The biopsies were cultured for 48 hours at 37°C in a humified mixture of 5% CO_2 in air. Following culture PGE_2 and LTB_4 were measured in the biopsy tissue and in the culture medium by radioimmunoassay, and the total amount expressed per mg of protein in the biopsy (ng/mg protein).

Smoking history appeared to have a biphasic effect on eicosanoid production, in that recent abstainers (< 10 years) had a greater PGE_2 and LTB_4 production (83.6 and 4.2 respectively) compared with never smokers (44.3 and 4.2); while long term abstainers (> 10 years) had reduced production (21.2 and 2.7 $p > 0.05$). Smoking abstention appeared to have a greater effect in the control group, increasing in recent ex-smokers (73.8 and 3.8) and decreasing in long term ex-smokers (19.7 and 2.7) compared with never smokers (28.8 and 4.0). A similar pattern was seen in patients with UC, but to a lesser extent.

Discontinuation of cigarette smoking increases the risk of ulcerative colitis, with the onset of disese approximately five years after abstention. The present study indicated that eicosanoid production increases following smoking cessation, and then decreases as abstention continues. Therefore, stimulation of eicosanoid production by stopping smoking may be the stimulus for the onset of ulcerative colitis in susceptible individuals.

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61 CIGARETTE SMOKING INFLUENCES EICOSANOID PRODUCTION BY THE COLONIC MUCOSA IN A DOSE DEPENDENT MANNER

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Cigarette smoking is an important factor in the pathogenesis of inflammatory bowel disease (IBD), with smoking being positively associated with Crohn's disease (CD); and negatively associated with ulcerative colitis (UC), with the proposal that smoking protects against UC.

Inflammatory bowel disease is characterised by chronic and acute inflammation of the intestinal mucosa and submucosa, with resultant chronic diarrhoea. Investigation of the mediators responsible for perpetuating the inflammatory response have shown that eicosanoids, particularly prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), are elevated, especially in the active phase of the disease. Cigarette smoking has been shown to inhibit PGE_2 synthesis by the gastric mucosa, while alveolar macrophages from smokers have reduced capacity to synthesis both PGE_2 and LTB_4. The aim of this study was to determine the effect of smoking habit on in vitro eicosanoid production by the intestinal mucosa in patients with IBD and controls.

Pinch biopsies from the colonic mucosa were obtained from 51 patients with IBD, of whom 9 were current smokers; and 95 patients in the control group, which contained 30 smokers. The biopsies were cultured for 48 hours at 37°C in a humified mixture of 5% CO_2 in air. Following culture PGE_2 and LTB_4 were measured in the biopsy tissue and in the culture medium by radioimmunoassay, and the total amount expressed per mg of protein in the biopsy (ng/mg protein).

Smoking had a biphasic effect on the eicosanoid production, in that light smokers (<10 cigarette per day) had enhanced PGE_2 and LTB_4 production (210.5 and 4.8 respectively); while medium smokers (10-19/day) had reduced production (27.6 and 3.7), which was enhanced in heavy smokers (>20/day), the difference reached significance (22.8 and 2.3) compared with non-smokers (44.3 and 4.2 $p<0.05$). The effects of smoking on eicosanoid production were more pronounced in patients with IBD, reaching significance in both light smokers (431.6 and 7.7 $p<0.05$) and heavy smokers (22.4 and 3.3 $p<0.05$) compared with non-smokers (59.4 and 4.8). The same trends were also seen in the control group, with light smokers producing more (54.4 and 4.8) and heavy smokers producing less (24.2 and 2.3) compared with non-smokers (28.8 and 4.0).

Cigarette smoking clearly has a profound effect on the aetiology of IBD, and the present study shows that heavy smoking inhibits eicosanoid production by the colonic mucosa, while light smokers may be exacerbating their condition by increasing their mucosal eicosanoid production.

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62 Effects of Smoking on Benzo (α) pyrene and Glutathione Metabolizing Enzymes in Man

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Smoking is the most important cause of lung cancer. We studied the influence of smoking habits on the metabolism of benzo (α) pyrene and glutathione in 190 patients with primary bronchial carcinoma. Seventy-two percent of these patients were current smokers, 18 % were former smokers, and 10 % were lifelong nonsmokers.

Benzo (α) pyrene (BP), like other polycyclic aromatic hydrocarbons (PAHs), needs activation to reactive metabolites to bind to cellular macromolecules and to exert adverse effects. Detoxification is preferably by conjugation with glutathione.

There were no significant differences in the activities of BP metabolizing enzymes in the lung tissue of smokers, former smokers, and nonsmokers. Only a tendency to slightly higher monooxygenase activities in smokers indicated an induction of these enzyme system to generate reactive metabolites. Glutathione and related enzyme activities showed a positive correlation to the number of cigarettes smoked daily. In contrast, the number of pack years and the duration of nicotine abuse had no effect either on PAH or glutathione metabolism.

These data indicate that the adverse effects of smoking may be based on a slight increase in the activity of cytochrome P450-dependent monooxygenases thus initiating the activation of BP to reactive metabolites. The correlation of the number of cigarettes smoked with the activities of the enzymes involved in detoxification is in favor of an increased requirement to remove reactive metabolites from the cell to prevent accumulation of toxic compounds. Thus detoxification in smokers has to be more effective than in nonsmokers to prevent early malignant transformation. As soon as detoxification becomes less effective due to an impairment of cellular defense mechanisms with age, an excess of nicotine abuse or other factors, a disturbance in the delicate balance between the generation and detoxification of reactive metabolites may result. This will facilitate tumor development.

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